GULF WAR VETERANS: LINKING EXPOSURES TO ILLNESSES

HEARING

BEFORE THE

SUBCOMMITTEE ON NATIONAL SECURITY, VETERANS AFFAIRS, AND INTERNATIONAL RELATIONS

OF THE

COMMITTEE ON GOVERNMENT REFORM

HOUSE OF REPRESENTATIVES

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GULF WAR VETERANS: LINKING EXPOSURES TO ILLNESSES

WEDNESDAY, SEPTEMBER 27, 2000

HOUSE OF REPRESENTATIVES,
SUBCOMMITTEE ON NATIONAL SECURITY, VETERANS AFFAIRS, AND INTERNATIONAL RELATIONS,
COMMITTEE ON GOVERNMENT REFORM,
Washington, DC.

The subcommittee met, pursuant to notice, at 10 a.m., in room 2247, Rayburn House Office Building, Hon. Christopher Shays (chairman of the subcommittee) presiding.

Present: Representatives Shays, Sanders, and Schakowsky.

Also present: Representative Metcalf.

Staff present: Lawrence J. Halloran, staff director and counsel; J. Vincent Chase, chief investigator; R. Nicholas Palarino, senior policy advisor; Robert Newman and Kristine McElroy, professional staff members; Alex Moore, fellow; Jason M. Chung, clerk; David Rapallo, minority counsel; and Earley Green, minority assistant clerk.

Mr. SHAYS. I'd like to call this hearing to order, this hearing of the Subcommittee on National Security, Veterans Affairs, and International Relations of the Government Reform Committee, which is conducting a hearing entitled, "Gulf War Veterans Linking Exposures to Illnesses."

Doubts remain, and may always remain, about the role of battlefield toxins and medicines in causing Gulf war veterans' illnesses. Today, we continue our oversight of the statutory process established to resolve those doubts in favor of sick veterans seeking proper diagnosis, effective treatment and fair compensation for their war-related injuries.

Embodying a recommendation made by this subcommittee, the Gulf War Veterans Act of 1998 directs the Department of Veterans Affairs [VA], not to wait for scientific certainty, but to look for any plausible association between presumed exposures and subsequent ill health. If credible evidence for the association is equal to or outweighs the credible evidence against, the VA Secretary is authorized to presume the illness is service related for purposes of health care eligibility and compensation determinations.

The National Academy of Sciences' Institute of Medicine [IOM], recently completed a study of peer-reviewed research on four of the agents of concern to Gulf war veterans: Sarin, pyridostigmine bromide [PB], depleted uranium [DU], and vaccines against anthrax and botulinum toxin. The IOM report now under review by the VA

suggests the difficulty and the urgency of linking presumed toxic exposures with chronic health effects.

Not surprisingly, medical literature to date contains little evidence to support any association between low doses of the agents in question and long term illnesses.

Those findings say far more about the stunted scope of scientific inquiry over the past decade than about the likely weight of scientific evidence. The significance of the report lies in the fact the IOM found virtually no evidence that would rebut a presumption of a causal association between these agents and many of the maladies suffered by Gulf war veterans.

As the IOM panel noted, the task of establishing plausible dose-response relationships was made more difficult by the lack of hard data on wartime exposures and by the lack of adequate military medical records.

Based primarily on studies following the Tokyo subway attack, the committee did conclude sarin exposures inducing immediate, if moderate, symptoms could also cause longer term health effects similar to those observed in many Gulf war veterans. But veterans' illnesses could not be more firmly associated with sarin because battlefield medical surveillance did not distinguish between the acute symptoms of mild sarin toxicity and the myriad of other environmental and stress-related health effects suffered by U.S. service personnel.

The IOM committee was also hampered by lack of access to classified information held by the Department of Defense [DOD], on toxic agents in the war theater. In the course of our oversight, many have called for full access to DOD records on chemical and biological detections. Given the statutory mandate that VA search broadly for information on toxic exposures, the VA should join us in pressing for declassification of all records relevant to the health of Gulf war veterans.

Doubts remain. But our obligation to act now on behalf of those willing to make a certain and timeless sacrifice can be subject to no doubt, no delay. They earned the benefit of any doubt about the extent of our debt to them. They should not be asked to wait for certainty that might come too late, if at all.

Mr. Metcalf is joining us from the great State of Washington, and I'd welcome any comment you'd like to make.

Mr. METCALF. Thank you very much. I do have a statement. Mr. Chairman, I want to thank you for the opportunity to once again be a small part of your courageous effort to answer questions regarding Gulf war illnesses and the vaccines used by our military personnel. Your determination to move forward and find answers has provided vital leadership for Congress on this critically important issue.

Indeed, we have an obligation to pursue the truth, wherever it may lead us. To do less would be to act dishonorably toward the dedicated men and women who stand between us and a still dangerous world.

For that reason, I have issued a report I would like to present to you and to the IOM committee culminating a 3-year investigation into the conduct of the Department of Defense with regard to the possibility that squalene, a substance in vaccine adjuvant for-

mulations not approved by the FDA, was used in inoculations given to Gulf war era service personnel. According to the GAO, General Accounting Office, scientists have expressed safety concerns regarding the use of novel adjuvant formulations in vaccines, including squalene.

The report reveals that the FDA has found trace amounts of squalene in the anthrax vaccine. The amounts recorded are enough to boost immune response, according to immunology professor, Dr. Dorothy Lewis of Baylor University. Therefore, my report concludes that, Mr. Chairman, you are absolutely correct in demanding an immediate halt to the current Anthrax Vaccination Immunization Program.

My report further states that an aggressive investigation must be undertaken to determine the source of the squalene and the potential health consequences to those who have been vaccinated, both during and after the Gulf war.

The report also documents at length DOD, Department of Defense, stonewalling attempts to resolve the squalene issue, which GAO investigators characterized as a pattern of deception. I think that's very significant. The GAO stated that the Department of Defense denied, denied conducting extensive squalene testing before the Gulf war, then admitted it after being confronted with the public record.

The DOD denied conducting extensive squalene testing before the Gulf war and then admitted to it after being confronted with the public record. I think that's significant. The GAO revealed that Department of Defense officials deliberating deployment of the anthrax vaccine expressed a "willingness to jump out and use everything," that's a quote, in discussing the experimental vaccines containing adjuvants not approved by the FDA.

GAO also found Peter Collis, Department of Defense official, who headed vaccine efforts, refused to cooperate with them. The report states that the Department of Defense has refused to act in good faith upon the GAO recommendations to replicate the findings of a test developed by renowned virologist, Dr. Robert Garry of Tulane University, although Department of Defense admitted that they could easily do so. The work of the Tulane researchers has been peer reviewed in a scientific publication of high standing.

Finally, my report states that Congress should take immediate action to review the findings of the GAO and the Armed Services Epidemiological Board and provide independent oversight for the immediate implementation of their recommendations. The board called upon the DOD to engage in close cooperation with the Tulane researchers.

Congress must get to the bottom of the labyrinth that has become known as Gulf war illnesses. Mr. Chairman, you have been in the forefront of this effort. As I am about to leave the Congress, I just want to once again commend you for your courage in this leadership role. Please stay the course. Veterans, active service members and their families deployed around the world are counting on you.

Thank you very much.

[The prepared statement of Hon. Jack Metcalf follows:]

JACK METCALF
2d District, Washington

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CHAIR, REPUBLICAN HOUSING OPPORTUNITY CAUCUS

REPUBLICAN POLICY COMMITTEE

Statement of Congressman Jack Metcalf
Subcommittee on National Security, Veterans Affairs, and International Relations
September 27, 2000

Mr Chairman, I want to thank you for the opportunity to once again be a small part of your courageous effort to answer questions regarding Gulf War Illnesses and vaccines used by our military personnel. Your determination to move forward and find answers has provided vital leadership for this Congress on this critically important issue.

Indeed, we have an obligation to pursue the truth, wherever it may lead us. To do less would be to act dishonorably toward the dedicated men and women who stand between us and a still dangerous world.

For that reason, I have issued a report culminating a three year investigation into the conduct of the DOD (Department of Defense) with regard to **the possibility that squalene, a substance in vaccine adjuvant formulations not approved by the FDA, was used in inoculations given to Gulf War era service personnel.** According to the GAO (General Accounting Office), scientists have expressed safety concerns regarding the use of novel adjuvant formulations in vaccines, including squalene.

The report reveals that **the FDA has found trace amounts of squalene in the anthrax vaccine. The amounts recorded are enough to "boost immune response**," according to of immunology professor Dr. Dorothy Lewis of Baylor University. Therefore, my report concludes that, Mr Chairman, you are absolutely correct in demanding an immediate halt to the current AVIP (Anthrax Vaccination Immunization Program).

My report further states that an aggressive investigation must be undertaken to determine the source of the squalene, and the potential health consequences to those who have been vaccinated, both during and after the Gulf War.

The report also documents at length DOD "stonewalling" attempts to resolve the squalene issue, which **GAO investigators characterized as "a pattern of deception**." The GAO stated the DOD denied conducting extensive squalene testing before the Gulf War, then admitted it after being confronted with the public record. The GAO revealed that DOD officials deliberating deployment of the anthrax vaccine expressed a "willingness to jump out and use everything," in discussing experimental vaccines containing adjuvants not approved by the FDA.

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Finally, my report states that "Congress should take immediate action to review the findings of the GAO and the Armed Services Epidemiological Board, and provide independent oversight for the immediate implementation of their recommendations." **The board called on the DOD to engage in close cooperation with the Tulane researchers.**

Congress must get to the bottom of the labyrinth that has become known as "Gulf War Illnesses." Mr Chairman, you have been in the forefront of this effort. As I am about to leave the Congress, I just want to once again commend you for your courage in this leadership role. Please stay the course. Veterans, active service members and their families deployed around the world are counting on you. Thank you so much.

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Executive Summary

Congressman Jack Metcalf has issued a report culminating a three year investigation into the conduct of the DOD (Department of Defense) with regard to **the possibility that squalene, a substance in vaccine adjuvant formulations not approved by the FDA, was used in inoculations given to Gulf War era service personnel.** According to the GAO (General Accounting Office), scientists have expressed safety concerns regarding the use of novel adjuvant formulations in vaccines, including squalene.

The report reveals that **the FDA has found trace amounts of squalene in the anthrax vaccine. The amounts recorded are enough to "boost immune response**," according to immunology professor Dr. Dorothy Lewis of Baylor University. Therefore, the report concludes that immediate action should be taken to halt the current AVIP (Anthrax Vaccination Immunization Program). It further states that an aggressive investigation must be undertaken to determine the source of the squalene, and the potential health consequences to those who have been vaccinated, both during and after the Gulf War.

The report also documents at length DOD "stonewalling" attempts to resolve this issue, which **GAO investigators characterized as "a pattern of deception**." The GAO stated the DOD denied conducting extensive squalene testing before the Gulf War, then admitted it after being confronted with the public record. The GAO revealed that DOD officials deliberating deployment of the anthrax vaccine expressed a "willingness to jump out and use everything," in discussing experimental vaccines containing adjuvants not approved by the FDA.

GAO also found Peter Collis, DOD official who headed vaccine efforts, refused to cooperate with them. The report states that the DOD has refused to act in good faith upon the GAO recommendation to replicate the findings of a test developed by renowned virologist Dr. Robert Garry of Tulane University, although DOD admitted they could easily do so. The work of the Tulane researchers has been peer-reviewed in a scientific publication of high standing.

Finally, the report states that "Congress should take immediate action to review the findings of the GAO and the Armed Services Epidemiological Board, and provide independent oversight for the immediate implementation of their recommendations." **The board called on the DOD to engage in close cooperation with the Tulane researchers.**

Congressman Metcalf believes it is clearly within the oversight responsibility of the Congress to get to the bottom of the labyrinth that has become known as "Gulf War Illnesses." **We have an obligation to pursue the truth, wherever it may lead us**. To do less would be to act dishonorably toward the dedicated men and women who stand between us and a dangerous world, willing to die if necessary to defend our nation.

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The Request For Investigation

- **August 29, 1997 Congressman Jack Metcalf requested the General Accounting Office (GAO) investigate reports that the presence of antibodies for squalene had been discovered in the blood of some sick Gulf War-era veterans.** The assay (test) being used to detect the antibodies had been developed at Tulane University by Dr. Robert Garry, world renowned virologist.(Appendix 1)

  At the time of Congressman Metcalf's request, the research by Drs. Garry, Asa and Cao had not yet been published in a peer-reviewed scientific journal. Their work, "Antibodies to Squalene in Gulf War Syndrome," was published in the February 2000 issue of *Experimental and Molecular Pathology*. (Appendix 2)

  NOTE: Squalene is a component of adjuvant formulations used in some experimental vaccines but not in any licensed vaccines. Squalene is found in shark liver oil, some vegetable oils, and the human liver and can also be manufactured through chemical engineering. (GAO/NSIAD-99-5).

Section One
The Investigation: A Pattern of Deception

- **September 1997 - March 29, 1999 General Accounting Office (GAO) investigators initiated their study and completed the report "GULF WAR ILLNESSES: Questions About the Presence of Squalene Antibodies in Veterans Can Be Resolved"** (GAO/NSIAD-99-5). The investigation was significantly slowed by government officials withholding or presenting incomplete information, ***leading GAO investigators to document their concerns questioning a "pattern of deception."*** (1) The following six dated entries are found in the background material for the GAO report. They illustrate the pattern of deception that clouded the investigation.

  November 14, 1997 GAO entrance conference with Department of Defense (DOD) officials. GAO notes state,
  1) "They said DOD had not performed or sponsored any research on synthetic or natural squalene or squalane until after the Gulf War. The sponsorship was through two CRADAs [Cooperative Research and Development Agreement]. However, they could not tell us who the CRADA's were with, what stage they were in, or what tests had been performed.
  2) "Squalene was used in two experimental adjuvants, after the war and involving fewer than 100 subjects. These were for HIV and Malaria vaccines. They said NIH had also used in some of their research protocols. DOD officials also stated that DOD was involved after animal testing stage." (2)
  *In background papers, GAO investigators stated, "However, GAO found evidence of several other studies in our searches of publication databases, references and articles. **Various DOD officials gradually acknowledged on a piece meal basis that their clinical research had started before the war,** that they had conducted 5 clinical studies with squalene and had planned a sixth, that the size of these studies was increasing and now has involved 572 human subjects, and that some of these studies were purely their own investigational New Drug (IND) Studies. **Moreover they had conducted numerous animal studies, particularly to develop a modern vaccine for anthrax. In fact, in most cases they only admitted to conducting research after we had discovered it in public records. On three occasions people attending a meeting did not report their own research on squalene adjuvants."** (3)*

  December 10, 1997 GAO entrance conference with Food and Drug Administration (FDA) officials. GAO investigators noted that it was a very productive meeting and recorded:

1) "The purpose of developing new adjuvants, even though alum is safe, is to use fewer inoculations, get a better response, and to check unconquered antigens. Earlier adjuvant ran into problems in animal testing... Most of DOD's work has been with Ribi Detox for malaria. **Their person most interested in developing own adjuvants at WRAIR** [Walter Reed Army Institute of Research] **is Carl Alving.**
2) "Allied had concerns about the quality of our vaccines. Michigan had some manufacturing problems.
3) "Karen is sure DOD used plague vaccine. They pushed it. She confirmed that squalene was used in placebos.
4) "FDA testing of drugs and vaccines: Good Manufacturing Practices inspection every 2 years. Test each lot released. No routine random sample. For bot tox [botulism toxoid] they also checked for safety and sterility, but not the makeup of the compound. DOD should have reserve samples. Required to have them for each lot. **Squalene should not be there**." (4)
NOTE: See Appendix 25 regarding the discovery by FDA in 1999 of trace amounts of squalene found in limited testing of Anthrax Vaccine, Adsorbed in the lots tested.

March 30, 1998 **GAO interview with Donald Burke, Director of AIDS research for DOD during the Persian Gulf War.** GAO recorded, "Burke said he was involved with AIDS trials at time of war and purposely chose not to get involved in BWD [biological weapons defense] issues at that time... In his AIDS work he experimented with MF59 [an adjuvant containing squalene] because alum was destructive to HIV proteins. He has had good cooperation with NIH [National Institutes of Health]. He recounted various studies, including a large one with 300 subjects getting MF59. . . He suggested we talk to . . . Carl Alving about DOD adjuvant research." (5))
GAO investigators noted, "Don Burke the former director of DOD's HIV research and Debbie Birx, the current director disagreed on the existence of a large early HIV trial with squalene with over 600 volunteers. She said he was thinking of an NIH trial. However, NIH reported no trials of that magnitude. (6)

April 6, 1998 **GAO interview with Dr. Carl Alving, DOD's top adjuvant researcher.** GAO stated,
1) **"Alving opened by saying he didn't know anything about Operations Desert Storm and Desert Shield (ODS) and the vaccines that were used. He is a researcher, and an expert, but not in the policy loop**.
2) "GAO pressed why he was not consulted about gulf war inoculations given his world class expertise. He admitted that just prior to gulf war he was asked if he could develop an anthrax vaccine on a crash basis. He stated that WRAIR has manufacturing capability, Ft. Detrick does not. He could have done it in 3-6 months but never received a follow on phone call to formally authorize the work. If asked, he could have done it but would have recommended MF59 for anthrax because Chiron had the manufacturing capacity and the desire to market it. Ribi, Chiron and Hunter

were the adjuvant leaders at the time. . . He was subsequently asked again (by DOD?) to develop an anthrax vaccine using liposomes, but it and all others. . . tested failed to protect monkeys with a single shot, which he thought was an absurd criteria. But he thought commercial considerations may have driven the criteria.
3) "He also said that as the world's foremost expert on lipids he knew quite a bit about cholesterol and its precursor, squalene. He doubted that a vaccine with squalene would produce a meaningful antibody response.
4) "Analysis: Overall, the commercial links appear to be crucial to the course of DOD vaccine R&D" (7))
GAO investigators recorded the following observation in a section titled, DOD officials were less than forthcoming about their role in Gulf War vaccine decision making: *"Carl Alving, DOD's top adjuvant researcher was not included in our meetings at WRAIR where he worked, nor even mentioned as someone we should interview. However, both NIH and FDA had said he was the person at DOD most involved with adjuvants. We subsequently met and while he acknowledged that he was probably the army's best expert on adjuvants, he at first denied having any role in the gulf war vaccine deliberations. After Kwai Chan left, Sushil Sharma pressed him on this, asking how could it be that they would discuss these issues without their principle expert. He then remembered that he had been called by someone from the army's biological warfare defense program at USAMRID [United States Army Medical Research Institute of Infectious Diseases], who asked if he could develop a new, more potent anthrax vaccine on a crash basis to use in the Operation Desert Shield. He worked on it and thought he could do it, but no one ever called him back. He wouldn't say who called from USAMRID or why he just didn't return the call."* (8))

April 19, 1998 Interview with Dr. Anna Johnson-Winegar, Director Environmental and Life Sciences, key participant in the tri-service committees advising on the science and vaccine production issues.
1) "Project Badger. [Tri-Service Task Force established prior to the Gulf War, (9/90) to investigate ways to increase production of biological warfare vaccines.] Badger was a discussion about the scientific issues involved in improving troop vaccine coverage. Discussions were wide-ranging and interesting, e.g. nonspecific immune enhancements, but there was not much data. Carl Alving was our in-house adjuvant expert, and a participant in our discussion. [Dr. Alving first told GAO he did not have any role in the gulf war vaccine deliberations, then minimized his involvement.] We discussed using liposomes, but they didn't have enough. You have to go to war with what you have, not novelties that don't have your full confidence.
2) " Adjuvants discussion and recommendations. Discussion of adjuvants was limited. Its one thing to discuss interesting phase 1 research, quite another to apply it to short term shortages. In the long run they can be of potential use. But scientific inference doesn't lead to immediate military operations. Some in the group were willing to jump out and use everything. (She refused to say who.) Our group advised

the Surgeon General who in turn worked with the JCS. There was not any data on what happens to people getting the anthrax and botulism vaccines at the same time. But we had to do it.
3) "Safety issues. There was little discussion of long term safety issues. They were thinking short term and immediate. Generally inactive vaccines don't have a problem. They used inactive antigens. But there were a lot of discussions regarding GMP [Good Manufacturing Practice] issues. For instance, they had trouble finding the exact same fermenter. Getting approval for a new one could take FDA 30 months. They went ahead started production with it and got retroactive approval. Anthrax vaccine is stable for up to 20 years if kept at right cool temperature." (DI-9)
NOTE: In a DOD Badger document File 120396_sep96_decls10_0002.txt, Subject: Desert Shield Biological Warfare HOC Working Group, the following statement is found:
"It was reported that the individuals from logist ___ USAMRIID, were expected back from theater today with the ___ anthrax and botulimum vaccines, antitoxin, ribavirin and centoxin. While in theater the items were under refrigeration; however, there was a report that the refrigerator failed to operate for a period of time and possibly these items were damaged. The items will be re ___ to USAMRIID and a determination made with regard to the disposition." (Appendix 3)

GAO notes state, "Anna Johnson-Winnegar played a major role in Project Badger, leading the effort seeking the urgent assistance of vaccine manufacturers. She sat in on most of the Project Badger meetings addressing BW defenses. Our interview with her revealed several contradictions. At first she said they had limited discussion about adjuvants, but then added that discussions were wide ranging and interesting, e.g. nonspecific immune enhancements, but there was not much data to base a decision. Alving, she said, was their in-house adjuvant expert, and a participant in their discussions. Some in the group felt is was one thing to discuss interesting Phase 1 research, quite another to apply it to short term shortages, ***but others were willing to jump out and use everything. She declined to tell us who advocated pushing forward the use of experimental vaccines.*** *" (10)*

April 23, 1998 **GAO meeting with General Ronald Blanck, Surgeon General of the Army, a discussion on the deliberations, decision making of DOD on vaccine production and administration for the Persian Gulf War.** GAO summarized Gen Blanck's recollection:

1) "One manufacturer, Michigan for both botulism and anthrax vaccine. We had a fair amount of anthrax vaccine but only a small amount for botulism (BT). However, we found Iraqis might have F and G strains so we contracted with Porton to make them. To best of his knowledge none were administered. We got it but didn't use it. Everything we used was from Michigan. Salk at Swiftwater had the capacity to help produce, but got nothing from them. He got NIH to approve NCI use.

2) "Novel Adjuvants Use. Blanck recalled no discussion of boosting immunogenicity with novel adjuvants. He was certain nothing was added to the products at Michigan. They decided to not do anything outside of the FDA. The anthrax vaccine used alum as an adjuvant.
3) "Who else should GAO interview. We should talk to Winnegar and Collis as planned. **Collis headed oversight for Badger and vaccine efforts**..." (11)

The following GAO statement summarized the failed attempts to interview Peter Collis. ***"Peter Collis, the chairman of the tri-service task force, Project Badger, repeatedly declined to talk to us.*** *First he said he could not meet unless he had the classified project summary to ensure his recall was accurate. We said we could provide those. Then he said as a civilian without a clearance he could not look at the notes. [GAO could proceed with process to obtain a temporary clearance for him.] Then he called declining one last time saying he really didn't know much. However, the Project Badger notes clearly show him to be at the hub of all the discussions, and that he conducted the briefings about the committees recommendations."* (12)

- **September 11, 1998** **GAO exit Conference with DOD officials.** GAO investigators record:
1) "We presented a summary our principal findings of our job on Squalene and Gulf War Illnesses, 713014. DOD officials stated that **if the independent researchers have developed a good test for squalene antibodies, there was no reason to wait for publication. The researchers could share it with DOD and they could cooperate on further research and development concerning squalene and Gulf War illness**. This could be done through a CRADA which would protect the rights of the independent researchers. DOD would like to validate the test, particularly its specificity.
2) " DOD officials again acknowledged that they had the know how to develop such an assay and could have tested for squalene antibodies but did not... They stated that DOD could do the screening for antibodies to squalene for veterans who are ill along with a larger battery of tests, but they would have to think through the health administration consequences because they didn't want to do screening if they were not prepared to act on the results. Colonel Takafuji concluded that the questions raised by the independent researchers are going to come back to DOD." (13)

Section Two
The Stonewalling and Obfuscation

- **March, 1999** **GAO presented to Metcalf their findings (GAO/NSIAD-99-5)**. GAO recommended DOD not wait for the peer-review and publication process, but take immediate action to: **"conduct research designed to replicate or dispute the independent research results** that revealed the presence of squalene antibodies in the blood of ill Gulf War-era veterans." Surprisingly, DOD's comments regarding the GAO recommendations, contained in the report, **accused GAO of being "scientifically and fiscally irresponsible," even though their own officials had stated there was no reason to wait for publication**. (14) The GAO report stated, "Safety concerns have been cited regarding the use of novel adjuvant formulations in vaccines, including squalene, and the associated adverse reactions. It has also been suggested that the safety of vaccines containing these formulations must be evaluated in conservative ways." (GAO/NSIAD-99-5 Page 3)

- **May 13, 1999** **Congressman Metcalf wrote Secretary of Defense William Cohen challenging DOD's refusal to carry out the GAO recommendations, and encouraging DOD to get to the truth by doing the research necessary to validate or dispute the Tulane test results.** (Appendix 4)

- **May 24, 1999** **Dr. Carl Alving called Dr. Robert Garry of Tulane, and indicated his "purely scientific" interest in Dr. Garry's work.** Dr. Alving also asked to review a draft of the manuscript on anti-squalene antibodies which was subsequently published. Dr. Garry agreed to fax him a copy of the *in progress* work for his personal review, requesting that he not circulate the copy. **Dr. Garry was not made aware of Dr. Alving's intent to circulate the paper and publicly subject it to scathing reviews as published on the DOD website prior to publication.** (Appendix 5)

- **May 25, 1999** **Dr. Russell Wilson of Autoimmune Technologies, Tulane's exclusive licensee for the anti-squalene antibodies technology, sent a letter to Dr. Carl Alving sharing information, and offering to provide information regarding the ASA (anti-squalene antibody) assay and research with DOD.** (Appendix 6)

- **May 28, 1999** **Dr. Sue Bailey, Assistant Secretary of Defense for Health Affairs, provided GAO the DOD's final response to the March, 1999 report**. She stated, "Our position and the concerns expressed in our comments to the draft report have not changed . . . The test methods proposed by the investigators at Tulane University need to be reviewed and validated by other scientists." ***DOD would not take action until the peer-reviewed publication process was complete.*** (Appendix 7)

- **Summer 1999** **An anonymously written DOD memo was obtained by the defense team representing five young Marines at Twenty-Nine Palms who were being court-martialed for their refusal of the anthrax vaccine.**
The six page document entitled, "Issues Relating to Antibodies to Squalene" was a scathing review by Dr. Carl Alving and Dr. Matyas of the unpublished work of Dr. Garry and his colleague Dr. Pamela Asa. It discussed the phone calls of May 24 and 25 between Dr. Alving and Drs. Garry and Wilson. With absolutely no proof, it accused Drs. Garry and Asa of an apparent anti-military agenda. **It concluded by stating "There is an obvious need for independent in-house research by the Army to examine the issues and implications, if any, of antibodies to squalene." Attached was a chart detailing a three year study, with a total cost of $1,260,834.00**. (Appendix 8)

- **July 23, 1999** **Dr. Bailey responded to Metcalf's May 13, 1999 letter to Secretary Cohen**. Once again she commented, " The Department's position and concerns have not changed from those published as Appendix VI of the GAO report." (Appendix 9)

- **September 27, 1999** **Metcalf letter to Secretary Cohen.** Metcalf replied, " . . . because of your department's years of research in this area, I ask that you reconsider and proceed with the GAO recommendations. Your current position of waiting for the completion of the peer review and publication process does not recognize the vast amount of research that the DOD has already accomplished regarding adjuvant formulations containing squalene. **The men and women who served honorably and are suffering from Gulf War Illnesses deserve truthful answers and *immediate action.***"(Apdx. 10)

- **October 25, 1999** **Because of DOD's refusal to cooperate with GAO recommendations, Congressman Metcalf asked for congressional intervention.** With the help of Congressman George Nethercutt, the House Report to H.R. 2561, the Fiscal Year 2000 Department of Defense Appropriations Bill, included language instructing DOD to develop and/or validate the assay to test for the presence of squalene antibodies. This legislative action was signed into law by the President on October 25. (Appendix 11)

- **November 5, 1999** **Metcalf received a reply to his September 27 letter from Secretary Cohen**. While stating: "The Department's position has been consistent and remains unchanged," he went on to inform Congressman Metcalf that **a DOD investigator** has been funded to "pursue a study to determine the feasibility of developing a test for antibodies to squalene." (Appendix 12)
Although Secretary Cohen did not identify the DOD investigator, GAO discovered that DOD had awarded the study to Dr. Carl Alving. The project was not designed to replicate or dispute theTulane findings as had been recommended by GAO, but to develop a different means of testing for antibodies to squalene. (Appendix 13)

- **January 2000** **DOD provided some members of Congress a report titled, "Development and Validation of an Assay to test for the Presence of Squalene Antibodies."** It stated, "This Report has been prepared in response to a requirement of the 106th Congress, House of Representatives, Report 106-244, 2000 Department of Defense Appropriations Bill." It acknowledged that DOD had funded a DOD researcher to "determine the feasibility of developing a test for antibodies to squalene." **It did not suggest a collaborative effort with Dr. Garry and his colleagues at Tulane to save valuable time for those who are suffering from Gulf War Illnesses, even though the researchers at Tulane had expressed their willingness to assist.** (Appendix 14)

- **January 31, 2000** **Congressman Metcalf was joined by nine colleagues requesting DOD do an objective analysis** of "Antibodies to Squalene in Gulf War Syndrome" - the peer-reviewed article published in the February 2000 issue of *Experimental and Molecular Pathology* by Drs. Asa, Cao and Garry. **The question from Congress was clear, "Given the published article, it seems prudent to use the assay if it could help sick Gulf War era veterans. Do you agree?"** (Appendix 15)

- **February 25, 2000** **Congressman Metcalf sent a strong letter to Secretary Cohen asking for immediate action to remove misleading information from the DOD's official Anthrax Vaccination Inoculation Program (AVIP) website regarding the peer-reviewed, published article on squalene antibodies.** Earlier in the week, the information had been discovered, prior to receipt of the DOD's official reply to the January 31 letter. (Appendix 16)

- **February 28, 2000** **The official DOD response to the January 31 letter was delivered to Congressman Metcalf's office**. *Most of the information provided was based on a review of the early draft*, not the published study which included significant changes. The half-page critical analysis of the peer-reviewed article was anonymously written, with no indication of the author's professional credentials to conduct and provide the review. **DOD did not address the congressional question regarding the potential use of the assay to help sick Gulf War era veterans.** (Appendix 17)

- **March 3, 2000** **Congressman Metcalf challenged Secretary Cohen to halt the obfuscation campaign that DOD was waging concerning the issues surrounding antibodies to squalene research. Metcalf provided ample evidence to demonstrate his conclusion.**. (Appendix 18)

- **March 27, 2000** **On behalf of Secretary Cohen, Dr. Sue Bailey responded to Congressman Metcalf's February 25 and March 3 letters.** She acknowledged needed modifications on the DOD AVIP website to more objectively reflect the Tulane research. She also informed Metcalf that the Armed Forces

Epidemiological Board (AFEB) would convene a subcommittee of experts to review and critique the published article in response to Congressman Metcalf's March 3 letter. (Appendix 19)

- **June 2000** **An exchange of letters in Experimental and Molecular Pathology. Dr. Carl Alving and Dr. John Grabenstein submitted a critique of the Tulane research, and Drs. Asa, Cao and Garry co-authored the response**. The journal Editorial Note made the following statement: "New findings require confirmation within the bounds of comparability. This is as true for methodology as it is for the data produced from a particular study. This exchange of letters ...relates to methodology. **Drs. Alving and Grabenstein offer no data against the conclusions of Asa et al.** (Appendix 20)

- **August 10, 2000** **Congressman Metcalf was presented the DOD 'objective analysis' of the article "Antibodies to Squalene in Gulf War Syndrome" by an Armed Forces Epidemiological Board subcommittee of experts.** They concluded unanimously that the research reported in the paper does not support its claim that the laboratory test created by Dr. Garry at Tulane may identify persons ill with Gulf War Syndrome. **However, on the last page of the report, they state, " Whatever the paper's flaws and since the AFEB cannot exclude the remote possibility that the authors have identified a laboratory means of distinguishing persons with possible Gulf War Syndrome (GWS) from all others, replicability becomes the major unresolved issue...Therefore we recommended that a suitable test of replicability be done in cooperation with the authors..."** They go on to state, **" ... we are trying to ... get quickly and inexpensively to a more meaningful bottom line: does the ASA assay clearly, reliably and unequivocally distinguish people with GWS from all others, and, if so, with what specificity and sensitivity?"** (Appendix 21)

Section Three
FDA Testing Reveals Squalene in Anthrax Vaccine

For over a year, the DOD has been contracting with SRI International to test for squalene in vials of the anthrax vaccine preparations which have been and are being given to military personnel. For some time, DOD documents have made two claims regarding squalene:
1) The FDA verified that none of the vaccines used during the Gulf War contained squalene as an adjuvant; and
2) they have found NO squalene in their testing of anthrax vaccine lots. *(Appendix 13 and 22) Documents on the DOD AVIP website from SIR International confirm their tests revealed no squalene in the anthrax vaccine sent to them for analysis. (Example: Appendix 23)*

- **January 31, 2000 Congressman Metcalf wrote the FDA asking them to confirm the following DOD statement made to Congress**, "**The FDA verified that none of the vaccines used during the Gulf War contained Squalene as an adjuvant.**" (Appendix 24)

- **March 20, 2000 The FDA responded to Congressman Metcalf and provided their official position.** "In fact FDA did verify to the Senate Special Investigations Unit on July 23, 1997, in a telephone conversation with Committee staff of the SIU, not with DOD, **that neither the licensed vaccines known to be used in the Gulf War, nor the one investigational product known to have been used**, contained squalene as an adjuvant in the formulations on file with FDA."

Most importantly, the FDA closed their letter with the following statement: "Very limited testing of Anthrax Vaccine, Adsorbed, conducted by CDER in 1999 determined that there were only trace amounts of squalene in the lots tested ... *(Appendix 25)*

- **Dr. Dorothy Lewis of Baylor College of Medicine sent a letter to Congressman Metcalf explaining that the test used by FDA which found low levels of squalene in Anthrax vaccine samples is a "much more sensitive technique" than the one used by DOD.** ***(Why would DOD use a less sensitive test procedure?)***

  Dr. Lewis determined, ***"The real issue is whether squalene in parts per billion was added to the vaccine preparations given to the military, as well as whether this concentration of squalene could alter the immune response."***

  While acknowledging the need for research to respond to the findings, she stated, ***"it is possible that very small amounts of a biologically active product could induce an immune response, either to the molecule itself or it could boost immune responses to other agents in the mixture."*** (Appendix 26)

CONCLUSION

1. Despite numerous denials by the Department of Defense, FDA has found squalene in the Anthrax Vaccine in limited testing. This vaccine is still being forced upon our active military duty personnel. Immediate action must be taken to halt the current AVIP (Anthrax Vaccination Immunization Program) until this matter is resolved. Aggressive research must be undertaken to determine the source of the squalene, if it could alter the immune response, and the potential health consequences to those who have been vaccinated, both during the Gulf War, and as a result of the mandatory, force-wide AVIP.

2. The recommendation of the Armed Forces Epidemiological Board subcommittee that, "...a suitable test of replicability be done in cooperation with the authors..." mirrors the findings of the GAO over eighteen months ago-- "DOD should conduct research designed to replicate or dispute the independent research results that revealed the presence of squalene antibodies in the blood of ill Gulf War-era veterans."

3. Congress should take immediate action to review the findings of the GAO and the Armed Services Epidemiological Board, and provide independent oversight for the immediate implementation of their recommendations. The Department of Defense has wasted years in their determined effort to stonewall this issue. The researchers at Tulane are willing to work with DOD to pursue answers for those suffering from Gulf War Illnesses. Within a few months, and for a small investment of money, important knowledge will be acquired that may offer real hope. For the men and women who honorably serve this nation, there is no valid reason for further delay.

All footnotes are references to General Accounting Office (GAO) background working documents for GAO final report "Gulf War Illnesses: Questions About the Presence of Squalene Antibodies in Veterans Can Be Resolved, (GAO-NSIAD-99-5)March 1999.

1. DI-23
2. DI-2
3. DI-23
4. F-5
5. DI-20
6. DI-23
7. DI-7
8 DI-23
9. DI-9
10. DI-23
11. DI-8
12. DI-23
13. DI-13
14. DI-13

Bolding and italics added for emphasis.

Mr. SHAYS. Thank you, Mr. Metcalf, and I was just going to comment that you will be very missed. We have appreciated your interest not only in this issue but in so many others, and I was sorry when you announced you weren't running again and I just know whoever gets to return next year, they will certainly miss you, and I will just say whatever this committee has done on this issue, and they have done, has been shared equally with Mr. Sanders on this issue. He has been truly at the forefront, and I welcome him here and I welcome any statement he'd like to make.

Mr. SANDERS. Thank you very much. And as Jack Metcalf just said, you have played an outstanding role in keeping this issue alive on behalf of tens and tens of thousands of men and women who are suffering from Gulf war illness, and it has been a pleasure to work with you and I applaud you for your leadership.

Over the past 5 years you have worked diligently to hold members of the military establishment accountable for their actions and, most importantly, their inaction. You and I and others have worked closely to try to get the Congress and the administration to fund serious research into potential causes and cures for the diseases known as Gulf war illness and to push for compensation for those veterans who have contracted these diseases. I am sad to say that despite our efforts we have up to this date only had limited success. The findings of the IOM study that we are examining today only serves to remind us how far we have yet to go on this issue.

Some good news is that Chairman Shays and I worked very hard this year to secure 1.6 million in the defense appropriations bill for research into whether Gulf war illnesses is the result of low level multiple toxin exposures which manifests itself as a condition known as multiple chemical sensitivity. We will be playing an active role in making sure that this money goes for serious research into this area.

I notice that Dr. John Feussner is here and he'll be speaking later, and I look forward to his discussion, the clinical study done with doxycycline and what the status is of that report, which is also an area we've worked on.

Let me begin by stating how I approach the issue of Gulf war illness, and that is when this country asks men and women to serve in the Armed Forces and those men and women are injured, whether in body or in mind or in spirit, the Federal Government has an absolute, unquestionable obligation to make those people whole to the maximum medical and scientific extent possible. In addition, the Federal Government has an obligation to compensate those veterans fairly, not to argue with them every single day, but to give them the benefit of the doubt, and when it is clear that veterans have been injured during their service, we should not deny them compensation just because we cannot say which particular exposure or combination of exposures caused that injury. In my view, on all counts the Federal Government has failed and failed miserably with respect to Gulf war illness.

You know, one of the unanswered questions of our time, and I certainly don't have the answer, Mr. Chairman, is that this turning one's back on veterans has gone on in this country for so very long. It started at the very least in World War II when for years we ig-

nored the impact of radiation illness. It went to Vietnam, where veterans organizations had to struggle for years and years to get the VA to acknowledge the horrendous impact that Agent Orange had, and we're still struggling with that fight today, and look what we have to do with Gulf war illness. I don't understand it. I really do not understand why when we ask men and women to put their lives on the line, when they come home we fight them. We become the enemy that they—similar to the enemy they fought in battle.

Over 100,000 veterans have reported suffering from some combination of symptoms associated with the syndrome we call Gulf war illness. Certainly it is important that we exhaust every possible research avenue to find the cause and the cure but we should not hold up compensation of Persian Gulf war veterans who have very real illnesses, because we have failed either through incompetence, insufficient resources or lack of dedication, or just lack of scientific knowledge, to identify the specific toxic compound or compounds that are responsible. This is particularly true because the Pentagon's negligence in keeping adequate records of exposures in the Gulf theater may prevent us from ever finding a definitive answer.

As for the IOM study that we are reviewing today, I say with all due respect to the IOM that this study only confirms what most of us already knew. There is a dearth of research in peer-reviewed scientific literature on the long-term health effects of exposure to various toxins that our soldiers encountered in the Gulf war theater.

Let me just add something to that. When I used to hear the word "peer-reviewed" I thought that was the right thing. But since I have been involved in this issue, you know when I hear "peer-reviewed" what it often connotes to me is the people who do not know much about an issue who cannot come up with an answer in an issue will always tell us what other people are doing, cutting edge research, that it's not peer reviewed and the peer-reviewed research that we hear tells us we don't know anything, that's the good research, we don't know anything when people are doing breakthroughs, who are doing cutting edge stuff, is not peer reviewed, and that's a problem I have seen for many years in this issue, in this area.

As the IOM reported, the peer-reviewed literature contains inadequate or insufficient information to determine whether there is an association between Gulf war illness and exposure to depleted uranium, between Gulf war illness and pyridostigmine bromide, between Gulf war illness and low level exposure to sarin gas, between Gulf war illness and anthrax vaccine or other vaccines or combinations of vaccines. These findings do not come as a shock to me or anyone else who has followed this issue.

The reason we do not have this research is that the Federal Government and, in particular, the Pentagon has failed to keep faith with the men and women who served in the Gulf. They have dragged their feet and, were it not for the efforts of people like Chairman Shays and the Gulf war veterans themselves, the military long ago would have forgotten about this issue. There would not have been—there would not be a Gulf war problem today.

I do want to commend the IOM on their research recommendations. These track the approach Chairman Shays and I have been advocating. Instead of looking for one single toxin as the cause of Gulf war illness, we need to investigate the impact of the multiple, often low level exposures that Gulf war veterans experienced. As the IOM report states, this, "may provide a more realistic approach toward understanding veterans' health issues and may provide insights for preventing illnesses in future deployments."

Finally, Mr. Chairman, I want to express my concern that there is still not the will within the military to get to the bottom of this very real health emergency. In my view, it is time for the military to make available to properly cleared independent researchers—you know, if you go back to somebody who year after year tells you, gee, I don't understand the problem, gee, I don't have a cure for the problem, what do you do? You go to a doctor that says, well, I'm not 100 percent sure that I have it, but this is a breakthrough, we're working on this. And the good news is you and I know, because you have brought every serious researcher in the United States to this committee, there are some good people out there doing some breakthrough research. Let's put more emphasis on some of those people.

So I want to just applaud you, Mr. Chairman, and commend the veterans organizations for their persistence, and you and I will continue to work on this issue, I'm sure.

Jack, thank you very much for your work over the years.

[The prepared statement of Hon. Bernard Sanders follows:]

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DOMESTIC AND INTERNATIONAL MONETARY POLICY

COMMITTEE ON GOVERNMENT REFORM AND OVERSIGHT
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NATIONAL ECONOMIC GROWTH, NATURAL RESOURCES, AND REGULATORY AFFAIRS
HUMAN RESOURCES
CO-CHAIR: PROGRESSIVE CAUCUS

STATEMENT OF REP. SANDERS DURING THE HEARING OF THE SUBCOMITTEE ON NATIONAL SECURITY, VETERANS AFFAIRS, AND INTERNATIONAL RELATIONS ENTITLED "GULF WAR VETERANS: LINKING EXPOSURES TO ILLNESSES"

September 27, 2000

I want to begin my remarks by commending Chairman Shays for his determined efforts to keep the suffering of the men and women who served this country during the Gulf War from being swept under the rug. Over the past five years, he has worked diligently to hold members of the military establishment accountable for their actions, and more importantly their inaction. He and I have worked closely for many years to try to get the Congress and the Administration to fund serious research into potential causes and cures for the diseases known as Gulf War Illness, and to push for compensation for those veterans who have contracted these diseases.

I am sad to say that despite our efforts we have up to this date only had limited success. The findings of the Institute of Medicine (IOM) study that we are examining today only serve to remind us how far we have yet to go on this issue. Some good news is that Chairman Shays and I worked very hard this year to secure $1.6 million in the Defense Appropriations bill for research into whether Gulf War Illnesses is the result multiple low-level toxic exposures which manifests itself as a condition known as Multiple Chemical Sensitivity. We will be playing an active role in making sure that this money goes for serious research in this area.

Let me start by stating how I approach this issue of Gulf War Illness. When this country asks men and women to serve in the armed forces, and those men and women are injured, whether in body, mind, or spirit, the federal government has an absolute, unquestionable obligation to make those people whole to the maximum medical and scientific extent possible. In addition, the federal government has an obligation to compensate those veterans fairly. And when it is clear that veterans have been injured during their service we should not deny them compensation just because we cannot say which particular exposure or combination of exposures caused that injury. In my view, on all counts, the federal government has failed and failed miserably with respect to Gulf War Illness.

Over 100,000 veterans have reported suffering from some combination of symptoms associated with this syndrome we call Gulf War Illness. Certainly, it is important that we exhaust every possible research avenue to find the cause and cure. But we should not hold up the compensation of Persian Gulf War veterans, who have very real illnesses, because we have failed – either through incompetence, insufficient resources or lack of dedication -- to identify the specific toxic compound or compounds that are responsible. This is particularly true here because the Pentagon's negligence in keeping adequate records of exposures in the Gulf theater may prevent us from ever finding a definitive answer.

As for the IOM study that we are reviewing today, I say with all due respect to the IOM that this study only confirms what most of us already knew. There is a dearth of research in peer-reviewed scientific literature on the long-term health effects of exposure to the various toxins that our soldiers encountered in the Gulf War theater.

As the IOM reported, the peer-reviewed literature contains inadequate or insufficient information to determine whether there is an association between Gulf War Illness and exposure to depleted uranium, between Gulf War Illness and pyridostigmine bromide, between Gulf War Illness and low level exposure to sarin gas, between Gulf War Illness and anthrax vaccine or other vaccines or combinations of vaccines. These findings do not come as a shock to me or anyone else who has followed this issue.

The reason that we do not have this research is that the federal government and, in particular, the Pentagon has failed to keep faith with the men and women who served in the Gulf. They have dragged their feet and were it not for the efforts of people like Chairman Shays and the Gulf War veterans themselves, the military would long ago have forgotten all about this issue.

I do want to commend the IOM on their research recommendations. These track the approach that Chairman Shays and I have been advocating. Instead of looking for one single toxin as the cause of Gulf War Illness, we need to investigate the impact of the multiple, often low-level, exposures that Gulf War veterans experienced. As the IOM reports states this "may provide a more realistic approach toward understanding veterans' health issues and may provide insights for preventing illnesses in future deployments."

Finally, Mr. Chairman, I want to express my concern that there is still not the will within the military to get to the bottom of this very real health emergency. In my view, it is time for the military to make available to properly cleared independent researchers the information the Pentagon now has in classified form so that we can get a better understanding of what biological and chemical exposures our veterans encountered in the Gulf and the levels of those exposures. If the Pentagon is unwilling to cooperate, I suggest that perhaps it is time for Congress to intervene. Furthermore, while we continue to move forward on serious research, I believe that on public policy grounds we should legislatively provide for a service-connected presumption for those suffering from Gulf War Illness.

Thank you, Mr. Chairman.

Mr. SHAYS. Thank you, Mr. Sanders. Just before going on with our panel, I ask unanimous consent that all members in the subcommittee be permitted to place an opening statement in the record and the record remain open for that purpose. Without objection so ordered.

I ask further unanimous consent that all witnesses be permitted to include their written statements in the record and, without objection, so ordered.

And I also without objection ask that the gentleman's statement, Mr. Metcalf's statement, and report be included in the hearing record, and I will move to include it in the full committee hearing on anthrax next Thursday.

You have been patient. Thank you very much. We will call on Mr. Harold Sox—Dr. Harold Sox, excuse me—professor and chair, Department of Medicine, Dartmouth-HitchCock Medical Center, accompanied by Samuel Potolicchio, who is professor, Department of Neurology, the George Washington University Medical Center. As you know, gentlemen, we swear you in and then we will take your testimony. If you would please stand.

[Witnesses sworn.]

Please be seated. I thank our other two staff for standing up in case you're required to make a statement. Thank you for anticipating that. It's very thoughtful.

Dr. Sox.

STATEMENT OF HAROLD SOX, M.D., PROFESSOR AND CHAIR, DEPARTMENT OF MEDICINE, DARTMOUTH-HITCHCOCK MEDICAL CENTER, ACCOMPANIED BY SAMUEL POTOLICCHIO, M.D., PROFESSOR, DEPARTMENT OF NEUROLOGY, THE GEORGE WASHINGTON UNIVERSITY MEDICAL CENTER

Dr. SOX. Good morning, Mr. Chairman and members of the committee. My name is Harold Sox. I chair the Institute of Medicine Committee on Health Effects Associated with Exposures During the Gulf War, which released its report about 3½ weeks ago. I appreciate the opportunity to provide testimony to you today based on the findings of our report. And I am accompanied by Dr. Samuel Potolicchio, also a member of the IOM committee.

The genesis of our report was a request from the Department of Veterans Affairs asking the Institute of Medicine to study the available scientific evidence on potentially harmful agents to which Gulf war veterans may have been exposed. Congress subsequently mandated a similar study specifying 33 specific agents. Before going further, I want to clarify the scope of the committee's work lest there be any misunderstanding.

The committee, IOM committee, was charged with assessing the scientific literature about potential health effects of chemical and biological agents present in the Gulf war theater. The Department of Veterans Affairs will use the findings of the report as it sees fit as a scientific basis for developing a compensation program for Gulf war veterans. Our committee was not asked to examine whether a unique Gulf war syndrome exists or to evaluate the literature on Gulf war syndrome or illnesses. The committee was not asked to make judgments about individual veterans' level of exposure to the

putative agents, as there is a presumption of exposure for everyone who served in the Persian Gulf theater.

For the first study of the series, the Institute of Medicine chose to study the agents of most concern to the veterans who advised us: Sarin, pyridostigmine bromide [PB], depleted uranium, and the vaccines to prevent anthrax and botulism.

Because there had been very few published studies of Gulf war veterans, most of the studies that we examined were about exposures in occupational, clinical and healthy volunteer settings. The committee members carefully assessed each study's quality, limitations and applicability, but it relied upon the peer review system that precedes publication in scientific journals as well.

Let me begin with the nerve agent sarin. Relatively high doses of sarin can cause overstimulation of nerves and muscles within seconds or hours, creating symptoms such as severe cramping, difficulty breathing, twitching and heavy sweating.

All of these short-term effects are well-documented and our committee ranked the evidence as sufficient to establish causality, the highest level of evidence. The long-term effects of sarin are a very different story. The evidence is far more limited in quantity and is weaker.

Studies describing three different populations exposed to sarin, two involving victims of terrorist attacks in Japan and one involving industrial accidents in the United States, establish possible links to neurological and psychological symptoms that persisted for 6 months or longer after exposure. In one of these studies some symptoms were still present up to 3 years after exposure. In all three studied populations, however, the patients all had an immediate, intense, widespread acute reaction, typical of high levels of exposure to sarin. Among the symptoms that persisted over the long term in these individuals were fatigue, headache, blurred vision and symptoms of post-traumatic stress disorder. It's important to remember that people who had long-term symptoms had all experienced intense symptoms immediately.

Because we are dealing with only three studies and because we could not rule out explanations, other explanations for the effects, the committee categorized these findings as limited or suggestive of an association well shy of the evidence needed to establish a strong link, but clearly warranting further investigation. We recommend long-term research to track the health of victims of the sarin attacks in Japan, since controlled studies of them offer the best opportunity to see if sarin has long-term health effects.

Few, if any, veterans reported symptoms of acute exposure to sarin in the Persian Gulf theater. Therefore we concerned ourselves with possible effects of sarin in doses too low to cause the acute reaction.

Based on available evidence, we could not form a conclusion about an association between the long-term health effects and exposure to doses of sarin that are low enough so that immediate signs and symptoms did not occur. Yet research with nonhuman primates gives us a hint that low doses of sarin over a period of several days may create delayed neurological reactions. More research is needed to substantiate this single finding.

The second agent that we considered was the drug pyridostigmine bromide [PB]. There have been many studies of the short-term effects of PB. The committee judged this evidence to be sufficiently strong to demonstrate an association between exposure and the immediate onset of mild transient symptoms, a link seen consistently in many studies. Long-term side effects of PB are another story. There simply was not enough evidence to draw any conclusion about PB's long-term effects. In other words, we don't know if they occur and we can't be certain that they don't occur.

The author of one series of studies has suggested that PB, either alone or in combination with other chemicals, may be related to some chronic changes in nerve function reported by Gulf war veterans. However, weaknesses in the design of these studies, which include uncertainty about whether exposures occurred and a small number of affected subjects, made it impossible for us to decide if exposure to PB is associated with long-term nerve damage. We recommend further investigation of this issue using an improved study design.

The third agent was depleted uranium. Health effects of natural uranium have been widely investigated, mostly in occupational settings, principally workers in uranium processing mills. While these studies have shown that uranium either has no effect or only a small effect, our committee found weaknesses in many of these studies. We could not draw conclusions about exposure to uranium and death from a number of diseases, including lymphatic or bone cancer, nonmalignant respiratory disease and diseases of the liver and gastrointestinal tract.

We were able to arrive at more certain conclusions regarding two diseases, kidney disease and lung cancer. We concluded that there is limited evidence of no association between kidney disease and exposure to uranium. We based this conclusion on adequate consistent studies that showed good kidney function despite continuous exposure to uranium as it dissolved from uranium fragments embedded in body tissues.

Similarly, at low levels of exposure to uranium, we found limited evidence of no association between—with death from lung cancer. At higher levels of exposure, though, the evidence did not permit any conclusion about a relationship between uranium and lung cancer. We recommend followup research on veterans with embedded fragments of depleted uranium and other long-term studies.

Finally, our committee considered the vaccines given to prevent anthrax and botulism. Based on our review of the scientific literature, we concluded that the evidence is sufficient to demonstrate an association between these vaccines and subsequent short-term local and systemic effects similar to those associated with any vaccination. But when we sought evidence for more lasting effects, we didn't find any published, peer-reviewed studies that systematically followed subjects over the long term. This situation is not unusual as vaccines are seldom monitored for adverse effects over long periods of time.

Since troops usually receive several vaccines, often within a short span of time, some have questioned whether several vaccines in combination may have created a cumulative effect that would not occur with any single injection. Although we did find some research

on cumulative effects of combinations of vaccines, the shortcomings in these studies made it impossible for us to form a strong conclusion. We did decide that this evidence was inadequate to determine whether an association with long-term effects exist.

I have provided a brief overview of our report's findings. The IOM is beginning the second phase of the study, in which it will examine the literature on health effects of pesticides and solvents. This study is scheduled to be completed in 2002, as the committee must review a vast body of literature on these compounds. Plans for future IOM studies include completion of the studies of the remaining agents listed in the legislation. In addition, the IOM will update its studies and reports as new studies become available in the published literature.

Thank you. Dr. Potolicchio and I will be happy to respond to your questions.

[The prepared statement of Dr. Sox follows:]

Statement of

Harold C. Sox Jr., MD
Chairman, Institute of Medicine Committee on
Health Effects Associated with Exposures during the Gulf War
and
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Lebanon, New Hampshire

before the
House Subcommittee on National Security, Veterans Affairs,
and International Relations

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Statement of Harold C. Sox Jr., MD
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Health Effects Associated with Exposures during the Gulf War
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Good morning, Mr. Chairman and members of the committee. My name is Harold Sox. I am a professor and chair of the Department of Medicine at Dartmouth-Hitchcock Medical Center in Lebanon, New Hampshire. I chaired the Institute of Medicine Committee on Health Effects Associated with Exposures During the Gulf War, which released its report on Thursday, September 7th. I appreciate the opportunity to provide testimony to you today based on the findings of this report. I am accompanied by Dr. Samuel Potolicchio, a member of the IOM committee and Professor in the Department of Neurology at George Washington University Medical Center.

The genesis of the report was a request from the Department of Veterans Affairs, asking the Institute of Medicine to study the available scientific evidence on potentially harmful agents to which Gulf War veterans may have been exposed. Congress subsequently mandated a similar study listing 33 specific agents for study. Thousands of Gulf War veterans have experienced chronic, unexplained health problems, and are asking whether these agents might be responsible.

It is important to clarify the scope of the committee's work. The committee was charged with assessing the scientific literature regarding potential health effects of chemical and biological agents present in the Gulf War. The findings of the report will be used by the Department of Veterans Affairs as a scientific basis for developing a compensation program for Gulf War veterans. The committee was not asked to examine whether a unique Gulf War syndrome exists or to review or evaluate the literature on Gulf War syndrome or illnesses. Additionally, it was not asked to make judgments regarding the veterans' levels of exposure to the putative agents as there is a presumption of exposure for Gulf War veterans. For the first study of the series, the Institute of Medicine chose to study the agents of most concern to the veterans: sarin, pyridostigmine bromide (PB), depleted uranium, and the vaccines to prevent anthrax and botulism.

Because of the limited studies in Gulf War veterans, most of the studies that we examined involved exposures in occupational, clinical, and healthy-volunteer settings. We carefully assessed each study's quality, limitations, and applicability.

When it comes to the *long-term* health effects of these substances, the bottom line is we simply don't know enough to say whether there is a connection between exposure to these agents or combinations of agents and specific health outcomes that remain long after the exposure. At most, we found some very limited evidence that might suggest a possible connection with the nerve agent sarin. These effects, if they truly exist, occur in individuals whose dose was large enough to cause acute symptoms immediately after the exposure. It will take further research to explore this relationship.

Let's begin with the nerve agent sarin. It is so potent that as little as 100 milligrams — about two drops — can cause convulsions and death. As a gas, roughly 50 milligrams can be fatal. Lower doses can cause overstimulation of nerves and muscles within seconds or hours, creating symptoms such as severe cramping, difficulty breathing, twitching, and heavy sweating. In the more severe cases, these symptoms are widespread and affect many parts of the body.

All of these short-term effects are well-documented, and we ranked the evidence as *sufficient to establish causality*, the highest level of evidence. In part, this means many studies have strongly, repeatedly, and consistently linked these acute health effects and exposure to sarin, and that the greater the exposure, the greater the effect. But the long-term effects of sarin are a very different story. The evidence is far more limited and much weaker. Studies describing three different populations — two involving victims of terrorist attacks in Japan and one involving industrial accidents in the United States — linked neurological and psychological symptoms that persisted for six months or longer. In one of these studies, some symptoms persisted for up to three years, the longest that any of the subjects were followed. In all three study populations however, the doses of sarin were high enough to trigger an immediate, intense, widespread, and acute reaction. Among the conditions that persisted over the long term were fatigue, headaches, blurred vision, and symptoms of post-traumatic stress disorder. In other words, people who had long-term symptoms were the ones who had experienced intense symptoms immediately.

Because we are dealing with studies of only three populations here, and because we could not rule out other explanations for the effects, the committee categorized these findings as *limited or suggestive of an association* — well shy of the evidence needed to establish a possible link, but warranting further investigation. In this case, we recommend research to track the health of the victims of sarin attacks in Japan, since they provide the best opportunity for conducting controlled studies.

Based on available research, we could not form a conclusion about an association between long-term health effects and exposure to lower doses of sarin — low enough so that there were no immediate signs or symptoms. Yet, research with nonhuman primates gives a hint that low doses of sarin over long periods may create delayed, neurological reactions. More research is needed to substantiate this finding. We recommend that such studies be pursued.

The second agent we considered was the drug pyridostigmine bromide. It is routinely used to treat patients with myasthenia gravis, a disease that causes weakening of the muscles. PB does have side effects. It is known to cause mild, tolerable, and transient gastrointestinal and muscular symptoms. In the Gulf War, troops were given packets of PB tablets to take in advance of a chemical weapons attack in order to blunt

the effects of nerve agents. The recommended doses were lower than those commonly used by doctors to treat patients with myasthenia gravis.

There have been many studies of the short-term effects of PB, and the committee judged this evidence to be sufficiently strong to demonstrate an association between exposure and the immediate onset of mild, transient symptoms. Many studies have repeatedly and consistently supported this linkage. Long-term side effects of PB are another story. There simply was not enough evidence to draw any conclusion about PB's long-term effects. In other words, we don't know if they occur, and we can't be certain that they don't occur. One series of studies has suggested that PB, either alone or in combination with other chemicals, may be related to some chronic changes in nerve function reported by Gulf War veterans. However, weaknesses in the design of these studies, which include uncertainties about exposures and a small sample, made it impossible for us to decide if exposure to PB is associated with long-term nerve damage. We recommend further investigation using an improved design.

The third agent that we considered was depleted uranium. During the Gulf War, some tanks and munitions containing depleted uranium caught fire or exploded. As a result, a number of soldiers are likely to have inhaled or ingested uranium dust, although the intensity of the exposure is unknown. Flying fragments containing depleted uranium injured others, leaving fragments embedded in tissue.

In its depleted form, uranium is 40 percent less radioactive than in its natural state. Health effects of natural uranium have been widely investigated, mostly in occupational settings. While these studies have either shown no effect or a small effect as a result of uranium exposure, our committee found weaknesses in many of these studies. We could not draw conclusions about exposure to uranium and death from a number of diseases, including lymphatic or bone cancer, nonmalignant respiratory illness, and diseases of the liver and gastrointestinal tract.

But we were able to arrive at more certain conclusions regarding kidney disease and lung cancer. We concluded that there is *limited evidence of no association* between kidney disease and exposure to uranium. We based this conclusion on several adequate, consistent studies that showed good kidney function despite continuous exposure to uranium as it dissolved from uranium fragments embedded in body tissues. Similarly, at low levels of exposure, we found *limited evidence of no association* with death from lung cancer. At higher levels of exposure, though, the evidence did not permit any conclusion about the relationship to lung cancer. We recommend follow-up research on veterans with embedded fragments of depleted uranium and other long-term studies.

Finally, our committee considered the vaccines given to prevent anthrax and botulism. More than 150,000 U.S. troops received injections of these vaccines to protect them in the event of biological warfare. Based on our review of the scientific literature, we concluded that the evidence is *sufficient to demonstrate an association* between these vaccines and subsequent short-term local and systemic effects. The symptoms include redness and swelling at the site of injection, similar to those associated with any vaccination. But when it came to evaluating more lasting effects, we didn't find any published, peer-reviewed studies that systematically followed subjects over the long term. This situation is not unusual, as few vaccines have been monitored for adverse effects over long periods of time.

Since troops usually received several vaccines, often within a short span of time, some have questioned whether several vaccines in combination may have created a cumulative effect when any single injection did not cause a reaction. Although we did find some research on cumulative effects of vaccines, the shortcomings in these studies made it impossible for us to form a strong conclusion. We did decide that this evidence was inadequate to determine whether an association exists.

This is a brief overview of the report's findings. The IOM is beginning the second phase of this study, and it will examine the literature on the health effects of pesticides and solvents. This study will be completed in 2002 as there is a large body of literature on these compounds. Plans for future IOM studies include completion of the remaining agents from those listed in the legislation. Additionally, the IOM will conduct updates of the literature as new studies become available.

Thank you for your attention. My colleague and I will be happy to answer your questions.

Mr. SHAYS. You needed a cheat sheet like I had, Potolicchio, correct?

Dr. POTOLICCHIO. It's an Italian name. Just follow all the vowels.

Dr. SOX. Sorry, Sam.

Mr. SHAYS. Just trying to get back at all those tough medical names that you guys have. What we're going to do is we're going to start out with Mr. Sanders, I'm going to ask some questions, and then we've been joined—Ms. Schakowsky is here. I will recognize her third and then we'll go to Mr. Metcalf, who's not an official member of this committee, though he has all the rights to ask the same questions we will, but just then at the end. Mr. Sanders. And we're going to go 10 minutes. We'll do 5 and 5, roll over 5.

Mr. SANDERS. Thank you very much, Mr. Chairman. Let me start off by asking you the question. You say in your statement, Dr. Sox, that for the first study of the series the Institute of Medicine chose to study the agents of most concern to the veterans, sarin, pyridostigmine bromide, depleted uranium and the vaccines to prevent anthrax and botulism. Now isn't one of the problems that we have is that we're sitting in a lovely room here in Washington, DC, but the reality of life, when you're at war, is that it may not be just one—there may not be just one agent that impacts on you. For example, 23, you're sitting there, you're scared to death, sitting in the heat, that the next day there may be a nerve gas attack on you. Psychologically what does that do to you? Meanwhile, at some point during the theater you may have been exposed to sarin, you may have been given a pyridostigmine vaccine, you may have had anthrax, you may have been exposed to burning oil wells, you may have a genetic disposition, you may have come from a place in your whole life you didn't absorb a lot of chemicals, so you're more susceptible to multiple chemical sensitivity. So my life history going into that battle is very different say than Mr. Shays. And so you add all of those things together, isn't there a problem that we're not looking at the totality and the synergistic impact rather than sarin here, depleted uranium here? Isn't there more to it than just one possible agent, and isn't that lacking in the way we're approaching this problem?

Dr. SOX. Well, our—the answer to your question is yes. We need to be aware of the potential for interactions between different agents as well as potentially a person's past history of exposure and, in an ideal study, to try to look at the links between agents and combinations of agents. We would have a clear understanding of an individual's personal exposure history, both before and after service in a theater of war and then reliable information about subsequent health experiences, and then we would try to link those together and see if we can detect effects that would not be seen looking at a single agent. Most of the research on the health effects of the agents that we studied were on single agents. In fact, we found only one study in our search which suggested a possible link between two agents, one in which mice that were injected with PB were subjected to the stress of having to swim.

Mr. SANDERS. All that I am saying, and I have got a number of other questions, in the real world it is not just sarin, she's in the military, she's suffering trauma being there, and so forth and so

on, that's the reality. It's not just we're sitting in a laboratory and we give somebody some sarin.

No. 2, I want to make sure I understand exactly what your report says. Am I correct that you have not ruled out, not ruled out depleted uranium, pyridostigmine bromide, sarin gas, anthrax vaccine or multiple vaccines or some combination of these as the cause of Gulf war illness, you have not ruled them out?

Dr. SOX. Our study was to look at the linkage between these four exposures and health effects, both diseases that are in textbooks as well as diseases that are not in textbooks because they're not well understood, such as Gulf war illnesses, and while we didn't find any compelling evidence that these exposures do cause health effects, neither was the evidence strong enough to conclusively rule out that they were not present. The closest we came was kidney disease and lung cancer with depleted uranium.

Mr. SANDERS. OK. I know that your mandate was only to review the peer-reviewed scientific literature on links between certain toxins and the symptoms that many Gulf war veterans are experiencing. Clearly, though, you had to undertake some background research into the types of symptoms these veterans are experiencing and the extent of those symptoms in order to do this analysis, is that correct?

Dr. SOX. Yes, sir.

Mr. SANDERS. OK. Based on that background review, is it your medical opinion that in general Gulf war veterans are suffering from a physical illness or illnesses as opposed to what might be termed a psychological condition?

Dr. SOX. Again, our committee charge was not to establish existence of a Gulf war syndrome. We read the published literature on this subject in order to provide background for our study of these compounds and their possible health effects, both on unexplained Gulf war illnesses as well as other illnesses. So if you want my personal opinion as a physician, I would say that ever since the Civil War, veterans of combat have experienced unexplained symptoms, and there's a great deal of overlap as you look at the symptoms that they experience in war after war coming right down to the present. So there's no question in my mind but what veterans do suffer unexplained illnesses, but this is a personal opinion. It was not a judgment of our committee. We didn't look at that question.

Mr. SANDERS. In your medical opinion, based on the background research you did, in your own experience does the fact that over 100,000 Gulf war veterans out of a total of less than 700,000 soldiers who served in the Gulf war have some combination of these symptoms suggest to you that these conditions we refer to as Gulf war illness have a connection to service in the Gulf war? In other words, if you have 100,000 or more folks out of 700,000 who have come down with a variety of illnesses now, it could be absolutely coincidental?

Dr. SOX. Well, again you're asking me to express a personal opinion, which is somewhat more informed than the average physician, but I am not expressing an opinion based on the findings of our committee, and based on my personal reading of those articles, I think that there's a relationship between service in the Gulf war

and these unexplained illnesses, but that was not a subject of the study.

Mr. SANDERS. Based on your own personal experience.

Dr. SOX. My own personal reading of those articles.

Mr. SANDERS. I appreciate that. In your view, is it possible that we will never establish the precise cause of Gulf war illness other than to conclude that it has some connection to service in the Gulf war.

Dr. SOX. I don't know how to answer that, sir. We have a number of exposures still to study and I would not want to form a judgment about what those studies might find. I don't have an opinion on that.

Mr. SANDERS. My last question is: Would you please explain what steps you took, if any, to obtain data from the DOD? Were they cooperative; were they not cooperative? You apparently did not get to review the classified materials. Did you request to and do you have staff who have security clearances?

Dr. SOX. Well, we did not actively seek DOD documents. Our charge was to study the published peer-reviewed literature, and there's a history of several hundred years that states that reliance upon scientific reports that have undergone peer review forms a credible basis for forming scientific judgments. And DOD documents, they are not scientific reports and so—but to answer your question briefly, we did not seek them. We were not interested in the level of exposure of individual veterans because that's something that because of presumption of exposure exists.

So it wasn't part of our charge to study DOD documents, and we did not request them.

Mr. SANDERS. Mr. Chairman, thank you very much.

Mr. SHAYS. Let me, if you don't mind, not on anybody's time, but just ask Mr. Potolicchio if he would want to respond to any of those questions that you asked. Is that all right?

Mr. SANDERS. Sure.

Mr. SHAYS. And then you can followup.

Dr. POTOLICCHIO. I think Dr. Sox has answered the questions appropriately.

Dr. SOX. If you think I am not doing a good job, you will interrupt.

Mr. SHAYS. Let me say because you're both partners here, you had one statement, but I don't mind if a question is directed to one of you to have the other jump in either with a qualifier or with whatever. I'd like either one of you to respond to—first, I'd like to just make a point. I wrestle with the fact that in terms of criminal law you're presumed innocent until guilt is proven and not, at least in the United States, presumed guilty until proven innocent. But I have the feeling that veterans are basically sentenced guilty because they're ill and they're guilty with no help in sight, and I have this general view that's come about through so many hearings that because there isn't a proven study or something that documents, therefore they're not going to have the presumption of an illness caused by their experience in the Gulf and therefore they are not going to get the help, not because there isn't that connection but because we can't illustrate that in fact there is that connection. And I understand where you come from as doctors and I think you

understand where we come from as people who actually sent them off to war. And so I'm troubled by the fact that we still have a system that is not going to help our veterans and that maybe 20 years from now they will prove there was this connection but by then it will be too late.

So I don't have the same kind of patience that I think some people have. My understanding is that you have looked at sarin, you've looked at pyridostigmine bromide, you've looked at depleted uranium, and you're looking at vaccines that were intended to prevent, deal with anthrax and botulism, and it's my understanding that the committee—let me say this to you before I ask the specific question. It's also my sense that the bill we passed makes the presumption of exposure to 33 agents; in other words, that at least we're not going to debate about it and then allow—that is the keyword, "allow"—the VA to establish a presumption that the exposures are related to illness and they're going to look at what you all have done and they are going to come to some conclusion. It allows but does not require.

Now when you tried to establish the categories of association from previous IOM studies, you would first agree that in some cases you were hampered by the fact there weren't enough studies, is that correct?

Dr. SOX. Enough studies.

Mr. SHAYS. I'll start with you, Dr. Sox.

Dr. SOX. Well, there were not enough studies of a quality that allowed you to make a scientific conclusion, yes, sir.

Mr. SHAYS. But not necessarily related to war experience?

Dr. SOX. Well, there were very few studies related to war experience. Most of them are in other settings, yes, sir.

Mr. SHAYS. And none of these studies would enable you to deal with the isolated—all things being equal, you look at a particular agent and then you've come up with some conclusions, is that correct? In other words, everything else is frozen?

Dr. SOX. Most of them are isolated studies in which you looked at one exposure in isolation of others.

Mr. SHAYS. And so you would certainly acknowledge, as I think Mr. Sanders has pointed out, that all things aren't equal, all things aren't held constant, there's exposure potentially to something but there's also exposure to others?

Dr. SOX. Yes, sir.

Mr. SHAYS. Would you make any comment, Dr. Potolicchio?

Dr. POTOLICCHIO. Maybe just one brief comment and that is, for instance, if you take two of the agents that we're considering here, pyridostigmine and sarin, actually one of them is given in order to protect the individual from exposure to the other. So they are given, they're sort of given simultaneously, but one hopefully is going to be protective and there's scientific evidence to prove that's the case.

Mr. SHAYS. Did you look at any studies that tried to determine what would happen if someone took more than the required allotment of PB? For instance, I have this tendency if I am putting fertilizer on my lawn, at least I did, that if one bag was good, two bags was better and three bags would be really terrific and I ended up with a lawn that was totally dead, and I know for a fact from

our witnesses that we had some who took the pill far in excess of what was recommended, far in excess. They went through that same logic. Did you look at any study that would have helped you determine that?

Dr. POTOLICCHIO. There were, we know from—and there's clinical evidence that if you take a whole bunch of pyridostigmine, let's say hundreds of pills, that you're going to really get sick, vomit and know that you have taken it, and I think that clinical response at least, tells you that we better not take anymore.

Mr. SHAYS. You know that from just observation, but did you look at any studies? In your peer review that dealt with taking too many pills, not your intuitive sense. But did you, was that part of your reviews and what reviews did you do? I'd like to know specifically.

Dr. POTOLICCHIO. Well, there are case reports of people being overexposed to certain agents, particularly pyridostigmine, and they will have clinical signs. But were studies taken in a double blind fashion that, you know, we were going to see how much can a person take of the drug, just to see what the side effects are going to be? No.

Mr. SHAYS. No. The view—we have had extensive testimony from MDs that have said that once you've taken so many you open yourself up to exposures that you wouldn't have been opened up to before, and the question I'm asking you is have you looked at anything in that regard?

Dr. POTOLICCHIO. The only studies that look at large doses of pyridostigmine are those confined to myasthenics; in other words, myasthenics have taken relatively large doses of pyridostigmine over a long period of time and there really haven't been any long-term health consequences of that. But as far as acute exposure to very large doses, will pyridostigmine kill you basically? We know well that sarin in little drops will kill you, but pyridostigmine will not kill you.

Mr. SHAYS. That's not what I'm asking. See, if you had been on this side you would have been, you would have been exposed to what we were, and that was that we had—we'd start our hearings from sick veterans who would explain to us that they were given really no instructions on what to do with these pills and that they didn't take them for days and then they took a lot of them, and then we had researchers come in and say that the impact on your brain and what it does in terms of it opens up the potential for other illnesses, so—do you want to just jump in?

Mr. SANDERS. Mr. Chairman, perhaps you have a better memory than I do, but I recall that we had the pharmacologist from Maryland, Dr. Teet, I believe his name was, who if I remember correctly said that that there is evidence if you are—it's one thing to take PB before exposure to sarin, which is the goal of presumably what that benefit was, but that if you take PB after the exposure to sarin it has an extremely negative impact. That's my memory, and I was wondering if they had looked at that.

Dr. POTOLICCHIO. There is, there is evidence that that's true because, you know, sarin, remember sarin is an agent that irreversibly blocks your cholinesterase. So in other words, once you're exposed to it and that cholinesterase is basically crippled, therefore

if you take another anticholinesterase on top of it after having that acute exposure, obviously you're going to amplify that. That's true. I don't disagree with that.

Mr. SHAYS. The question I'm asking is was that part of your peer review?

Dr. POTOLICCHIO. The study that you're referring to is done only in animals. There is no evidence in humans that that kind of after exposure is going to lead to further compromise.

Mr. SHAYS. I still want an answer, though. It wasn't part of your peer review because there were no studies?

Dr. POTOLICCHIO. In animals.

Mr. SHAYS. But there were no studies in humans?

Dr. POTOLICCHIO. There are no studies in humans.

Mr. SHAYS. So it's not part of your peer review?

Dr. POTOLICCHIO. Correct.

Mr. SHAYS. So what am I supposed to conclude in that? And what I conclude, I think, is that it kind of relates to your observation about peer review, there's no peer review there, but I'll tell you what happened when your report came out. The press said there's no linkage, you've discounted and—but it's like not having all the facts, and this is what—you know, I know you're doing your best but the bottom line is what are we supposed to conclude.

Dr. SOX. Well, no evidence isn't the same as evidence of no effect.

Mr. SHAYS. Say that again.

Dr. SOX. No evidence is not the same as evidence of no effect. So clearly the press, if they concluded there was no effect, made a mistake.

Mr. SHAYS. I understand, but that's the reality.

Dr. SOX. Yeah.

Mr. SHAYS. Would you walk me through, and then I will go to my colleague, on the concept of sufficient evidence of a causal relationship, sufficient evidence of an association, limited suggested evidence of an association, inadequate, insufficient evidence to determine whether an association does or does not exist, and then limited suggested evidence of no association, so there are five categories. If you would walk me through those.

Dr. SOX. It will just take me a minute to find them.

Mr. SHAYS. Yeah, take your time.

Dr. SOX. First of all, the causal relationship. The evidence fulfills the criteria for sufficient evidence of an association; that is to say, all of the other levels of evidence, and satisfies several of the criteria that have been used to assess causality.

Mr. SHAYS. So that would be the most certain, you would have very little doubt there's evidence of a relationship?

Dr. SOX. Yeah, it is very hard to——

Mr. SHAYS. The causal relationship.

Dr. SOX. Yes, sir.

Mr. SHAYS. The cause and effect. The second one is sufficient evidence of an association.

Dr. SOX. And that states that there's been a positive association between an exposure and a health outcome in studies where other factors that might confuse the interpretation of that relationship can be ruled out with reasonable confidence, so that you think you

can focus just on the exposure and not on other factors that might lead to the same result.

Mr. SHAYS. The next one is limited suggestive evidence of an association.

Dr. SOX. Here there's, there is evidence of an association between an agent and health outcomes, but the strength of the conclusion that you can draw is limited because you can't be sure that other factors that might explain the results aren't present. So you might have four or five things that could account for the result, one of which is the exposure. You can't be sure that the other ones aren't there and accounting for at least part of the effect.

Mr. SHAYS. We have two more. Inadequate, insufficient evidence to determine whether an association does or does not exist, and I would assume that's neutral, you can't go either direction?

Dr. SOX. It doesn't change your thinking one way or the other. It's like there isn't any information.

Mr. SHAYS. But the first three lead you toward——

Dr. SOX. Uh-huh.

Mr. SHAYS. The last one is limited suggested evidence of no association. So we have those five. If you would just quickly tell me, sarin fit which category again?

Dr. SOX. Well, the acute effects of sarin were a causal relationship.

Mr. SHAYS. So that's the strongest you could have.

Dr. SOX. Yes, sir. And then there were long-term effects in people who experienced the acute effects and that came in the limited suggestive category.

Mr. SHAYS. OK. That was just one higher than neutral?

Dr. SOX. Inadequate, yes, sir, and then——

Mr. SHAYS. PB.

Dr. SOX. Just to finish on sarin, evidence for long-term effects in people who did not experience any short-term effects of sarin, there was just no information except the one study in primates, which obviously requires a lot of followup.

Mr. SHAYS. OK. And PB.

Dr. SOX. In PB, the evidence was sufficient of an association between PB and acute effects lasting pretty much during the day that you took it.

Mr. SHAYS. No long-term harm?

Dr. SOX. But in terms of long-term effects the evidence was inadequate to determine whether there was or was not an association.

Mr. SHAYS. But you didn't look at whether PB then opened the door for other illnesses with other agents? I mean, that's on the record, correct?

Dr. SOX. There wasn't, there weren't any studies that showed us that PB opens the door to other exposures causing, leading to illness, yes, sir.

Mr. SHAYS. Thank you, and depleted uranium.

Dr. SOX. Depleted uranium, with two exceptions, the evidence was inadequate to determine whether an association does or does not exist. The two exceptions were lung cancer and kidney disease and in those cases there was limited or suggestive evidence of no association.

Mr. SHAYS. OK. And then finally, vaccines to prevent anthrax and botulism?

Dr. SOX. There was sufficient evidence of an association between immunization or vaccination and acute effects lasting a day or two, the sort of thing that many of us in this room have experienced. But the evidence was insufficient, similarly, just wasn't there. The studies weren't there——

Mr. SHAYS. You couldn't determine one way or the other?

Dr. SOX [continuing]. To determine any long-term effects.

Mr. SHAYS. So that's a neutral issue?

Dr. SOX. Yes, sir.

Mr. SHAYS. Thank you very much, and, Ms. Schakowsky, I do appreciate your patience. Thank you.

Ms. SCHAKOWSKY. Thank you very much, Mr. Chairman. I haven't been here as long as the chairman or Mr. Sanders, but I have to tell you that in the hearings we have had regarding issues where we put our people in the Armed Services in harm's way and the kind of information we had, it has been very, very frustrating. It seems in some ways that the policy of our government is no news is good news or no findings are good findings or no studies are good studies. And I'm looking through your testimony, Dr. Sox, and I see words like "limited studies." Because of the limited studies in Gulf war veterans, when it comes to long-term health effects of these substances, the bottom line is we simply don't know enough on PB. There simply was not enough evidence to draw any conclusion about PB. In other words, we don't know long-term effects, if they occur, and we can't be certain if they don't occur. Weaknesses in the design of these studies made it impossible for us to decide.

When it came to anthrax and botulism, we've had lots of hearings on anthrax. When it came to evaluating more lasting effects, we didn't find any published peer review study. I'm saying pretty much what everybody has said already. This is not unusual. As few vaccines have been monitored for adverse effects over long periods of time. When it comes to combinations, you say the shortcomings in these studies made it impossible for us to form a strong conclusion, and I am wondering if we're going to go on for another 10 years, and I realize this isn't your fault.

I'm just trying to ask you what we can do about this. We come and say, well, someone studied your study and what they found was there wasn't enough information. We keep doing studies of studies that have been done that say there hasn't been enough study. So I'm wondering when we get down to doing some real study and what your recommendations would be so that next time we have a study we can come back with some real reports.

Dr. SOX. Well, the wheels of research grind slowly.

Ms. SCHAKOWSKY. Are they in process?

Dr. SOX. Pardon me.

Ms. SCHAKOWSKY. Are they in process?

Dr. SOX. Basically physicians have known about postwar syndromes, as I said, since the Civil War and, from my understanding, serious research into the cause of those syndromes really has only begun after the Persian Gulf war. So we're, in my opinion, at the beginning of serious, careful study of an important group of ill-

nesses that have existed for 100, nearly 140 years and it's going to take a while to accumulate good evidence.

LBJ declared war on cancer in 1968 and we have made a lot of progress in understanding the biology of cancer, but actually we're only now beginning to see some results or promise of some results from that research 30, 35 years later. I'm optimistic that we're starting on a process that's going to lead us to answers, but I don't expect the answers to come quickly.

Ms. SCHAKOWSKY. Well, inconclusive results of real clinical studies that happen, that's one thing, and research that's being done, but I'm just wondering what the protocols are, for example, if we had—we made a decision about how many anthrax vaccines, how many dosages we should give and etc., and then when we come back and say well, based on what, what is your knowledge of this, how do we know about its effectiveness and its side effects, short and, well, mostly long term, so at what point should we be doing these studies and I would say that, that with agent orange, I mean, we have known about these symptoms that result from exposures during wartime, but are we engaged directly in the kind of research right now, and if that's the case, I haven't really heard about it.

I mean, we heard when it came to anthrax all kinds of these voluntary reporting systems and no real answer as to how are we going to determine the effects.

Dr. SOX. Well I am not an expert on the current state of research on Persian Gulf-related illnesses. Dr. Feussner, who will be speaking to you shortly, I am sure can tell you what studies are being done.

Ms. SCHAKOWSKY. Thank you.

Mr. SHAYS. Thank you. Mr. Metcalf does not have any questions.

Mr. Sanders.

Mr. SANDERS. Thank you very much Mr. Chairman. As I indicated earlier because of the diligence of the chairman and his staff, we have had the opportunity on this committee to hear from, seems to me, some extraordinary researchers all over this country who have been doing breakthrough work, and there are a number of them, and I don't recall all of them, but I just was kind of curious, two names come to my mind, and I wonder if you can give me your views having reviewed their works.

Dr. Robert Hayley is with the University of Texas, and as I recall, not having his work in front of me, he is not ambiguous about his belief that exposures in the Gulf have resulted in brain damage, which are causing severe physical problems for Gulf war veterans, no ifs, ands, buts and maybes, that is his belief. What's your view on that?

Dr. SOX. Well, the committee carefully examined Dr. Hayley's work and had the opportunity to talk with Dr. Hayley about his work at one of our open sessions, and the committee ultimately concluded that there were difficulties with the design of Dr. Hayley's work that made it impossible to draw any conclusions at this point.

I think our bottom line would be that in a small population of veterans, Dr. Hayley has done some studies that generate interesting ideas and hypotheses about the biological basis for some of the

symptoms that people are experiencing, but until those studies are replicated by other investigators and larger more representative populations, the evidence that Dr. Hayley has produced is too weak for us to draw any conclusions upon which to base in our report.

Mr. SANDERS. Too weak in the sense that the number of veterans, the sampling was too small.

Dr. SOX. Well, the sampling was too small. He studied basically a group of symptomatic veterans, and he, using some statistical techniques, put them in the subgroups which seemed to have different combinations of symptoms, and then he looked at different measures of brain function comparing one group of sick veterans to another group of sick veterans. It's a pretty basic principle of epidemiologic research to include an unexposed control, somebody who never went into the Persian Gulf theater, and with the exception of a couple of more recent studies, he has not had unexposed controls, but even putting that aside, the history of science is that you don't rely on one study. You, somebody does a study, and then several people try to replicate it. Sometimes they succeed and then it becomes part of the body of scientific understanding, and sometimes they don't and it falls by the wayside and right now, I think Dr. Hayley's work is in the category of remains to be repeated by other investigators.

Mr. SANDERS. Are other people, to your knowledge, trying to replicate that?

Dr. SOX. I will have to ask Dr. Feussner to respond to that, I don't know.

Mr. SANDERS. What about Dr. Urnovitz.

Dr. SOX. Doctor who?

Mr. SANDERS. Urnovitz.

Dr. SOX. I don't know about his work. Sam, do you remember anything.

Dr. POTOLICCHIO. By name, I don't.

Mr. SANDERS. Don't know his name, no?

Dr. SOX. None of us.

Mr. SANDERS. Dr. Claudia Miller, peer review.

Dr. POTOLICCHIO. Claudia Miller I think—we know we've had exposure to Claudia Miller.

Dr. SOX. If I remember.

Mr. SANDERS. She's involved in multiple chemical sensitivity.

Dr. SOX. She gave us a presentation. We did not review the literature on multiple clinical sensitivities and really don't have a basis upon which to judge her work.

Mr. SANDERS. See Mr. Chairman, may I repeat a point I made earlier, what seems to happen, and I think Ms. Schakowsky was making this point, we review people who say I don't know the cause of Gulf war illness, I don't have a cure to Gulf war illness, that's peer review. The people like Hayley or Urnovitz or Miller who say, you know, I think we're on to something, I think there's something real here, those are rejected because apparently not enough people have peer-reviewed that, we push them aside. It would seem to me, and correct me if I'm wrong, given the fact that after—and I don't mean to be critical of you. I know you're just one part. We've had 100 people up here who keep telling us the same thing.

So we get a little bit frustrated, but when people come up here and they say I think we're on to something, it would seem to me that the logical reaction for Hayley's work or Urnovitz's work or Miller's work would be for people to jump up and down and say, thank God, we may have a breakthrough, why are we—are you recommending for example that resources now be devoted to replicate Hayley's work so that 5 years from now, we don't have people coming before us saying Hayley's work was interesting, but nobody's replicated it, so why don't we replicate it? Tell us that Hayley is wrong or he is right, or Urnovitz is wrong or is right.

Dr. SOX. You know the history of scientific enterprise is somebody comes up with a finding and then somebody funds studies to try to replicate that study. So the answer to your question is yes, if somebody comes in here and makes a claim of an important result, the answer should be to fund other investigators to replicate the result.

Mr. SANDERS. I agree with you but based on that I mean all that you told me about Hayley, Urnovitz, you've never heard of Hayley. You said there is nobody, you know, he's out there, we don't have enough evidence to suggest that he is right or wrong, but you should be coming in here and saying this guy is saying something that's significant, it's different to other people, he's claiming some results, either he's crazy or he's not, let's find out; true?

Dr. SOX. Well, yes and in our research recommendations, we called for work to replicate Dr. Hayley's findings.

Mr. SANDERS. One of the things that we can use—we have gone through this for 10 years, so what we would like people to say is look, there are some breakthroughs here, we cannot tell you at this moment whether these people are right or wrong, maybe they're wrong, let's find out and say that they're wrong, or if they are right, let's devote a whole lot of money to moving forward so we can use their research to develop a cure for Gulf war illness. I didn't hear you say that.

Dr. SOX. Did you hear me say it?

Mr. SANDERS. No, I didn't hear you say it.

Dr. SOX. Well——

Mr. SANDERS. For example, tell me now, based on all of your research, if you were the President of the United States, or better yet, if you were going to recommend to the President of the United States, Mr. President, we have got a problem and I, based on all of my research, advocate to you that you spend X dollars in the following areas because we have some promising breakthroughs, but we just don't know about it. What would you recommend to the President?

Dr. SOX. Well, I would recommend to the President a program of research to try to replicate some of the interesting results of investigators like Dr. Hayley, but I probably also call upon the President to establish a committee, to establish research priorities so we don't just focus on the areas where some scientists are working, but also going out and looking at areas where nobody has looked yet, perhaps for lack of funding, so in other words, we need a comprehensive approach to the study of postwar illnesses, and part of that approach is to followup on promising results of investigators like Dr. Hayley.

Mr. SANDERS. But that's where we were 10 years ago. You've studied all of the literature. So I am asking you, all right, give me, at this point, if you can, who are the people out there that you see are doing breakthrough work that, in fact, need help right now for additional funding so that we can determine whether they're right or whether they are wrong. Is Hayley one of them?

Dr. SOX. I don't know anything about Dr. Hayley's funding, but clearly, Dr. Hayley is studying veterans and coming up with some interesting results, but I'm not sure it's Dr.—that Dr. Hayley needs more money. It may be that other people need more money to followup on his studies and to take it to the next step.

Mr. SANDERS. That's fine I am not here defending Dr. Hayley. All I am saying is you've done a lot of research; you're a scientist we are not. You have studied the literature. Can you just tell us who are the people out there you are thinking that you think are doing breakthrough work that we should try to give more support to?

Dr. SOX. Well, the only name that comes to mind is Dr. Hayley. I do believe that the Baltimore group has been studying the veterans with depleted uranium fragments needs continued support but if—but I really don't think that I should be the person to tell you who ought to be funded. I think that's something for more deliberation.

Mr. SANDERS. In all due respect, I disagree with that. We need guidance. We are not scientists, you are, and what we need help on is for somebody to come before us and say look these guys have been doing this stuff for 10 years. It's going nowhere in a hurry, this is possible, this is potential we do need that kind of help Mr. Chairman.

Mr. SHAYS. The other place we need help is when you're looking at what studies are available and you realize there just aren't any peer review studies in certain areas. I'd like to—in general, I'd like to read one paragraph, then we're going to get on to the next panel, unless Ms. Schakowsky has any questions. But this is the paragraph on page 3. It's a fairly long one, but I am going to read it all to you. It starts out—it's kind of in the middle of the page.

All these short term effects are well documented, and we rank the evidence as sufficient to establish causation, the highest level of evidence. In part, this means—and we're talking about nerve agent sarin—in part, this means many studies have strongly repeatedly and consistently linked these acute health effects and exposures to sarin, and that the greater the exposure, the greater the effect, but the long-term effects of sarin are a very different story. The evidence is far more limited and much weaker.

Studies describing three different populations, two involving victims of terrorist attacks in Japan and one involving industrial accidents in the United States, link neurological and psychological symptoms that persisted for 6 months or longer. In one of these studies, some symptoms persisted for up to 3 years, the longest that any of the subjects were followed.

In all three studied populations, however, the doses of sarin were high enough to trigger an immediate, intense widespread and acute reaction. Among the conditions that persisted over the long term were fatigue, headaches, blurred vision and symptoms of post traumatic stress disorder. I might just say parenthetically, that's a very

common symptom for our veterans who have come before our committee. In other words, people who had long-term symptoms were the ones who had experienced intense symptoms immediately.

Now, I want—the keyword here is "intense." How did you define "intense?" Was it walking intense or drop dead intense? I mean, fall down intense? What defines "intense?"

Dr. POTOLICCHIO. The level of exposure was based only on clinical findings, and maybe one laboratory test when it was available. You know, there is no real exposure data on sarin in any of the Japanese populations. We don't know how much any individual got at any time. If you look at the reports and the way they were written up, there was a man that was 100 feet from the release of the gas in Matsumoto, Japan, and he opened the window of his room and that man eventually died in convulsions and respiratory arrest, and he was just a few hundred feet away, but he probably had a maximum exposure but nobody knows exactly how much.

Mr. SHAYS. I am just trying to understand.

Dr. POTOLICCHIO. The thing is that when you get to the clinical findings, you say, well, there has to be an intense—in other words, someone's had an exposure, he, at least, had some symptoms of exposure that we recognize and that would be the acute cholinergic syndrome.

Mr. SHAYS. I understand that.

Dr. POTOLICCHIO. Or the enzyme that you measure in the blood is depressed to such a degree——

Mr. SHAYS. Let me not get to that. Let me just get to your concept of "intense," and I want to relate "intense" into war. I mean, I can remember when I was being chased by some older kids who wanted to beat me up, I've never run so fast. I didn't even know that I was exhausted. I was so damn afraid. I ran across a highway without looking either direction, and as far as I was concerned, I was pretty healthy, but later I realized I was just, I was just totally—I was sore, I was always these things and I was sore when I was running, but I didn't know that. I didn't have people shooting at me. So I guess what I'm trying to determine is are you making an assumption that there was not an intense exposure in the Gulf because people didn't fall down or something?

Dr. POTOLICCHIO. We're not making an assumption about anything that happened in the Gulf war theater. We're saying if you have an exposure to sarin, you will have acute symptoms. Now whether or not you can identify those——

Mr. SHAYS. Describe those acute symptoms, please.

Dr. POTOLICCHIO. Well, your acute symptoms would be——

Mr. SHAYS. Would be fatigue, headaches, blurred vision, what?

Dr. POTOLICCHIO. No.

Mr. SHAYS. What would they be?

Dr. POTOLICCHIO. Your acute symptoms would be difficulty breathing, watery eyes, probably GI upset, in other words, gastrointestinal upset, your muscles might start to twitch, and you can actually go into a convulsion if the exposure is intense enough.

Mr. SHAYS. But not necessarily. All those symptoms I would wager our veterans have experienced in the Gulf, not all of them but a good number, blurred vision.

Dr. POTOLICCHIO. You wager they have been exposed to that?

Mr. SHAYS. We had testimony of people describing those very symptoms, not after but during. OK. So the symptoms you have described, just for the record I will state, was statements to us by veterans that they experienced in the theater, clearly. I think we're all set unless you have any, Ms. Schakowsky, any questions. Thank you all very much.

Our next witnesses are John Feussner, Dr. John Feussner sorry, chief research and development officer Department of Veterans Affairs accompanied by mark brown Ph.D. director environmental agents study, department of Veterans Affairs. Do you all have anybody else that would help you in any testimony? If so I would ask them to stand up. Thank you and if you're asked to then respond, we would check out the names. I ask you to raise your right hands please.

[Witnesses sworn.]

Mr. SHAYS. Note for the record that our witnesses have responded in the affirmative and Dr. Feussner, you will be making the statement, and Dr. Brown you would also be responding to questions. Thank you very much. Appreciate your patience.

STATEMENTS OF DR. JOHN FEUSSNER, CHIEF RESEARCH AND DEVELOPMENT OFFICER, DEPARTMENT OF VETERANS AFFAIRS, ACCOMPANIED BY MARK BROWN, Ph.D., DIRECTOR, ENVIRONMENTAL AGENTS SERVICE, DEPARTMENT OF VETERANS AFFAIRS

Dr. FEUSSNER. Mr. Chairman and members of the subcommittee, thank you for this opportunity to discuss the status of the current Federal research program on Gulf war veterans illnesses. Accompanying me today is Dr. Mark Brown, who is the director of the VA's Environmental Agents Service.

In your invitation letter, you indicated that the purpose of the hearing was to review the findings and recommendations of the recent Institute of Medicine report. You also requested a discussion of the plans for additional research by the IOM and a status report on other research on Gulf war veterans illnesses.

To date, the Federal Government is projecting cumulative expenditures of $151 million of Gulf war research from fiscal year 1994 through fiscal year 2000. There are over 192 projects at various stages of completion in the research portfolio on these veterans illnesses.

For the sake of brevity, Mr. Chairman, I will only summarize the research recommendation of the Institute of Medicine report and the response of the research working group.

With regards to sarin specifically, the IOM has recommended long-term followup of populations exposed to sarin in the Matsumoto and Tokyo terrorist attacks. The research working group concurs with the IOM recommendation.

The IOM recommends studies in experimental animals to investigate the long-term effects of acute, short-term exposures to sarin at doses that do not cause overt cholinergic effects. Since 1996, the DOD has funded nine toxicology studies focusing on the effects of sarin, alone or in combination.

In addition to the IOM recommendations on animal studies on sarin, the research working group is coordinating three epidemio-

logical studies that are focusing on the health of veterans potentially exposed to low level sarin due to the Khamisiyah demolitions, one at the Navy Health Research Center, a second at the Oregon Health Sciences University, and a third by the Medical Followup Agency of the Institute of Medicine.

In addition to the IOM recommendation on animal studies on sarin, the research working group also is coordinating a contract to the medical followup agency to perform an epidemiologic study of the long-term effects of short-term exposure to nerve agents in human volunteers in experiments conducted at the Aberdeen Proving Ground in the 1950's to 1970's.

With regard to pyridostigmine bromide, the IOM recommends research on chemical interactions between PB and other agents, such as stressful stimuli and certain insecticides. Since 1994, VA and DOD have funded 30 projects related to PB alone or in combination with other chemicals or stressful stimuli. One important and consistent result of recent studies is that stressful stimuli such as swimming, heat or restraint stress do not cause an increase in the permeability of the blood brain barrier or cause pyridostigmine bromide to cross the blood brain barrier into the brain.

The IOM recommends research on differences in genetic susceptibility that may contribute to increased risk of disease. VA and DOD have funded eight projects on genetic factors that may alter the susceptibility to the effects of PB or sarin.

Concerning vaccines, the IOM has recommended long-term systematic research to examine potential adverse effects of anthrax and botulinum toxoid vaccination in multiple species and strains of animals. The research working group concurs that long-term research is needed to examine potential adverse effects. Such research is underway in DOD laboratories. Also, the CDC, the Centers for Disease Control and Prevention, plans to fund nonhuman primate studies of the health effects and efficacy of the anthrax vaccine later this year.

The IOM has recommended identification of cohorts of Gulf war veterans and Gulf war era veterans for whom vaccination records exist. The CDC published a study of Air Force Gulf war veterans in 1998 which included measuring antibodies to anthrax and botulinum to determine which individuals had received the vaccines. The CDC found no relationship between the vaccinations and development of multisymptom illnesses.

Similarly, researchers in the United Kingdom have also published a study this year on a cohort of nearly 1,000 Gulf war veterans for whom vaccination records exist. There was no association between having received the anthrax vaccine and the development of multisystem illness.

The IOM has also recommended long-term longitudinal studies of the participants in the anthrax vaccine immunization program. In 1999, DOD funded a long-term longitudinal study of participants in the anthrax vaccine immunization program study located at the Naval Health Research Center.

Finally with regard to depleted uranium, the IOM recommended continued followup of the Baltimore cohort of Gulf war veterans with DU exposure. The research working group concurs with the recommendation. While the Baltimore clinicians have seen no de-

finitive evidence of adverse clinical outcomes associated with uranium exposure to date, the veterans who were involved in the friendly fire incidents will remain under continuing medical surveillance.

The IOM has recommended continued followup of the cohorts of uranium processing workers. The research working group concurs with this recommendation.

The IOM has recommended additional studies of the effects of depleted uranium in animals. DOD has funded five toxicology projects that are investigating the health effects of DU in experimental animals. For example, there was no detectable kidney toxicity in rats embedded with DU pellets, even at very high concentrations of urinary uranium.

Mr. Chairman, we know that combat casualties do not always result in obvious wounds and that some veterans from all conflicts return with debilitating health problems. VA recognizes its responsibility for developing effective treatments and prevention strategies for such illnesses.

Studies clearly show that some Gulf war veterans report chronic and ill-defined symptoms including fatigue, neurocognitive problems and musculoskeletal symptoms at rates that are significantly greater than nondeployed veterans.

Mr. Chairman, thank you again for permitting me this opportunity to summarize our work. You have my assurance that we will continue this effort to resolve, or at least ameliorate health problems in our patients to the greatest extent possible.

Mr. Chairman, I will conclude my testimony here and ask that you enter the entire written testimony into the record. I actually think you did that.

[The prepared statement of Dr. Feussner follows:]

Statement of
John R. Feussner, M.D.
Chief Research and Development Officer
Veterans Health Administration
Department of Veterans Affairs
Before the National Security, Veterans Affairs, and
International Relations Subcommittee
Committee on Government Reform
U. S. House of Representatives
Regarding
Research on Gulf War Veterans' Illnesses

September 27, 2000

**

Mr. Chairman and members of the Subcommittee, thank you for this opportunity to discuss the status of the current federal research program on Gulf War veterans' illnesses. I serve as the Department of Veterans Affairs' (VA) Chief Research and Development Officer and the Chairperson of the Research Working Group (RWG) of the Persian Gulf Veterans Coordinating Board (PGVCB). Accompanying me today is Dr. Mark Brown who is the Director of VA's Environmental Agents Service.

In your invitation letter, you indicated that the purpose of the hearing was to review the findings and the recommendations of the recent Institute of Medicine (IOM) report, *Gulf War and Health, Volume 1.: Depleted Uranium, Sarin, Pyridostigmine Bromide, Vaccines*. You also requested a discussion of the plans for additional research by the IOM, and a status report on other research on Gulf War veterans' illnesses, both underway and completed.

As you know, the United States deployed nearly 700,000 military personnel during the Gulf War from August 1990 to the cease-fire on February 28, 1991. Within months of their return, some Gulf War veterans reported various symptoms and illnesses that they considered to be connected to their war-time service. Veterans, their families,

and the VA have been concerned about possible health effects from exposures during the Gulf War, including the anti-nerve-agent drug pyridostigmine bromide, depleted uranium, vaccines, and chemical warfare agents.

Overview of the Research Portfolio on Gulf War Veterans' Illnesses

To date, the Federal government is projecting cumulative expenditures of $151 million for Gulf War research from FY 1994 through FY 2000. There are over 192 projects at various stages of completion in the research portfolio on these veterans' illnesses. In FY 1999 and FY 2000, 42 new projects have been added to this portfolio. Research projects have been funded in the categories of basic research and applied research, such as clinical epidemiology and population-based epidemiologic research. To date, 83 federally funded projects have been completed. All projects and their focus areas are described in detail in annual reports that are submitted to Congress each year.

IOM Report: *Gulf War and Health, Volume 1.*

Background on the IOM Report

The Under Secretary for Health sent a letter to the National Academy of Sciences Institute of Medicine (IOM) on October 31, 1997 requesting an IOM study. The purpose of the study was to comprehensively review, evaluate, and summarize the published peer reviewed scientific literature regarding the associations between various Gulf War exposures and adverse health effects experienced by some Gulf War veterans. The IOM was also requested to make recommendations for additional scientific studies to resolve areas of continued scientific uncertainty related to health consequences of Gulf War service. On June 24, 1998, VA signed a contract with the IOM for a 27-month study, at a total cost of $1.25 million.

This effort was modeled after the successful process VA has used since the early 1990s to establish compensation policy for Vietnam veterans exposed to Agent Orange.

Four months later, in October 1998, Congress supported this effort with legislative mandates, including the "Veterans Programs Enhancement Act of 1998"

(Pub.L. No. 105-368) and the "Persian Gulf War Veterans Act of 1998" (Pub. L. No. 105-277). The contract with IOM meets the requirements of these Acts.

The IOM reviewed the scientific and medical literature on the adverse health effects associated with exposure to sarin, pyridostigmine bromide, vaccines, and depleted uranium. The review took into account the strength of scientific evidence and the appropriateness of the scientific methods used to identify associations. It includes an assessment of biologic plausibility that these exposures are associated with illnesses experienced by Gulf War veterans. In many cases, the data distinguished differences between transient and long-term health effects, related to the dose of the exposure. Therefore, IOM reported separate findings on the potential transient, short-term effects of each exposure, as well as the potential long-term effects. As required by P. L. 105-277 and P. L. 105-368, the Department is currently evaluating the IOM report to determine whether or not a presumption of service connection is warranted for any illness related to the exposures covered in the report.

A major strength of the study is that in planning its work, the IOM committee asked representatives of veterans service organizations for advice in setting its priorities. Veterans advised the committee to begin the project by reviewing these specific risk factors. Therefore, this report looked at the exposures that were of greatest health concern to veterans themselves. The IOM report should provide some reassurance to veterans and their families about these health concerns.

Findings and Recommendations of the IOM Report and
Response of the Research Working Group

Sarin:

- IOM Findings on potential long-term effects of sarin: IOM concluded that there was limited or suggestive evidence of an association between "exposure to sarin at doses sufficient to cause acute cholinergic signs and symptoms and subsequent long-term health effects." IOM concluded that there was inadequate evidence to determine whether an association does or does not exist between "sarin at low doses insufficient

to cause acute cholinergic signs and symptoms and subsequent adverse long-term effects."

- Basis for IOM Findings on potential long-term health effects of sarin: IOM stated that, after human exposures to sarin at doses high enough to cause poisoning symptoms, numerous chronic effects have been reported. These health effects have been observed in industrial workers accidentally exposed to sarin in the U.S. and in the two terrorism attacks in Japan. IOM noted that "there are no well-controlled studies of long-term health effects in humans exposed to sarin at doses that do not produce acute signs and symptoms."

- IOM Recommendations and Research Working Group Response:

1. Long-term follow-up of populations exposed to sarin in the Matsumoto and Tokyo terrorist attacks.
    - The RWG concurs with IOM's recommendation that Japanese scientists should continue the long-term follow-up of populations exposed to sarin in the Matsumoto and Tokyo terrorist attacks. We plan to keep apprised of the results of these studies.

2. Studies in experimental animals to investigate the long-term effects of an acute, short-term exposure to sarin at doses that do not cause overt cholinergic effects and minimal acetylcholinesterase inhibition.
    - Since 1996, DoD has funded several studies of the long-term effects of short-term sarin exposure at doses that do not cause overt symptoms and cause only minimal acetylcholinesterase inhibition. Nine toxicology studies are focusing on the effects of sarin, alone or in combination. These combinations have included PB, DEET, permethrin, chlorpyrifos, heat stress and/or exercise stress.

3. In addition to the IOM recommendation on animal studies on sarin, the RWG is coordinating three epidemiological studies that are focusing on the health of veterans potentially exposed to low-level sarin due to the demolitions at Khamisiyah. The results of one of these projects were published in 1999 (project DoD-1B). The conclusion was

there were no differences in rates of health problems among Gulf War veterans, who were potentially exposed to subclinical levels of sarin, compared to Gulf War veterans who were not exposed. The second Khamisiyah-related project is being performed by the Oregon Health Sciences University (DoD-63). The purpose is to compare neurological symptoms and results of neurobehavioral tests between Gulf War veterans, who were potentially exposed to low levels of sarin, versus Gulf War veterans who were not exposed. The third Khamisiyah-related project is being performed by the Medical Follow-Up Agency (MFUA) of the IOM (DoD-69). The purpose is to compare self-reported health problems between Gulf War veterans, who were potentially exposed to low levels of sarin, versus Gulf War veterans who were not exposed.

4. In addition to the IOM recommendation on animal studies on sarin, the RWG coordinated a contract for MFUA to perform an epidemiologic study of the long-term effects of short-term exposure to nerve agents in human volunteers in experiments conducted at Aberdeen Proving Ground in the 1950s to 1970s (DoD-93).

5. Research on genetic factors that may alter susceptibility to sarin toxicity.
    - VA and DoD have funded a number of research projects on genetic factors that may alter the susceptibility to sarin and/or PB toxicity. These studies are described in detail in the section on PB below.

Pyridostigmine Bromide (PB):

- IOM Findings on potential long-term effects of PB: IOM concluded that there was inadequate evidence to determine whether an association does or does not exist between PB and long-term adverse health effects.

- Basis for IOM Findings on potential long-term health effects of PB: IOM noted that no reports of chronic toxicity were available related to human PB exposure in clinical or military populations. IOM reviewed two studies of PB use in Gulf War veterans, and concluded "the epidemiological data do not provide evidence of a link between PB and chronic illness in Gulf War veterans."

- IOM Recommendations and Research Working Group Response:

1. Research on chemical interactions between PB and other agents such as stressful stimuli, and certain insecticides.

    - Since 1994, VA and DoD have funded 30 projects related to PB, alone or in combination with other chemicals or stressful stimuli. In particular, VA and DoD have funded 18 projects on the potential interactions between PB and other agents. Five of these projects have published results, focusing on the effects of PB in rodents, in combination with DEET, permethrin, swimming stress, restraint stress, or exercise stress (projects VA-49, DoD-10, DoD-37, DoD-62, DoD-65). One important and consistent result of recent studies is that stressful stimuli, such as swimming stress or restraint stress, do not cause an increase in the permeability of the blood-brain barrier, or cause PB to cross the blood-brain barrier into the brain. In 1996, the earliest research in this area was performed, which indicated increased permeability of the blood brain barrier to PB, due to swimming stress in a particular strain of mice. Several more recent studies have failed to replicate this finding using a variety of species, types of stressful stimuli, and extremely high doses of PB.

2. Research on differences in genetic susceptibility (e.g., genetic polymorphisms of butyrylcholinesterase or paraoxonase) that may contribute to increased risk of disease.

    - VA and DoD have funded eight projects on genetic factors that may alter susceptibility to the effects of PB or sarin, including polymorphisms of enzymes. Four projects in humans are evaluating the effects of genetic differences in polymorphisms of acetylcholinesterase, butyrylcholinesterase, and/or paraoxonase (projects DoD-21, DoD-60, DoD-65, DoD-112). Two projects in humans are evaluating the effects of gender and weight (project DoD-11, DoD-64). Two projects in rats are evaluating the effects of genetic differences in polymorphisms of acetylcholinesterase and butyrylcholinesterase (VA-5D, VA-49).

3. Epidemiological studies on the possible long-term health effects of PB.

- The RWG concurs with IOM that neurologists, who perform long-term follow-up of the course and treatment of myasthenia patients, should consider the possible long-term effects of PB. These patients take PB for many years. IOM concluded that PB has been used safely and effectively in thousands of myasthenia gravis patients since the 1950s. However, there has not been a systematic evaluation to determine if there are subtle long-term effects. We plan to keep apprised of the results of such long-term studies of myasthenia gravis patients, and have instituted contacts on this issue with the Myasthenia Gravis Foundation of America.

Vaccines:

- IOM Findings on the potential long-term effects of vaccines: IOM concluded that there was inadequate evidence to determine whether an association does or does not exist between anthrax vaccination, botulinum toxoid vaccination, or multiple vaccinations, and long-term adverse health effects.

- Basis for IOM Findings on potential long-term health effects of vaccines: IOM stated there were no published, controlled studies of the long-term effects of anthrax vaccination or botulinum toxoid vaccination. IOM reviewed only a few studies of the long-term effects of multiple vaccinations, which were too limited to draw conclusions.

- IOM Recommendations and Research Working Group Response:

1. Long-term systematic research to examine potential adverse effects of anthrax and botulinum toxoid vaccination in multiple species and strains of animals.

    - The RWG concurs that long-term research is needed to examine potential adverse effects of anthrax and botulinum toxoid vaccination in experimental animals. Such research is underway in DoD laboratories. Also, CDC plans to fund non-human primate studies of the health effects and efficacy of the anthrax vaccine in late 2000.

2. Identification of cohorts of Gulf War veterans and Gulf War era veterans, for whom vaccination records exist, followed by careful studies of current symptoms, functional status, and disease status.

- The Centers for Disease Control and Prevention (CDC) published a study of Air Force Gulf War veterans in 1998, which included measuring antibodies to anthrax and botulinum to determine which individuals had received the vaccines. The CDC found no relationship between the vaccinations and the development of a multisymptom illness (chronic symptoms of fatigue, cognitive and mood problems, and musculoskeletal pain).
- The United Kingdom has also published a study in 2000 on a cohort of 923 Gulf War veterans for whom vaccination records exist. There was no association between having received the anthrax vaccine and the development of multisymptom illness, as defined by CDC.

3. Long-term longitudinal studies of the participants in the Anthrax Vaccine Immunization Program that would actively monitor and systematically collect and analyze data about symptoms, functional status, and disease status.

- In 1999, DoD funded a long-term longitudinal study of participants in the Anthrax Vaccine Immunization Program. The Naval Health Research Center is establishing DoD-wide surveillance of hospitalizations in military hospitals, linking these to data on anthrax vaccine recipients (project DoD-99). This active surveillance system ensures early detection of any associations between vaccinations and severe reactions that require hospitalizations. In addition, there are several ongoing projects that are following smaller groups of vaccine recipients to evaluate adverse effects. In Chapter 7, IOM summarizes several of these smaller completed and ongoing human studies, nearly all of which are unpublished. IOM strongly urges the DoD investigators who are conducting these studies to submit their results to peer-reviewed journals for publication. Additionally, IOM recently started a new two-year study on the safety and efficacy of the anthrax vaccine, funded by DoD. This new study will review some of the unpublished, non-peer reviewed information that was not previously available.

Depleted Uranium (DU):

- IOM Findings on the potential long-term health effects of DU: IOM concluded that there is limited or suggestive evidence that there is no association between exposure to uranium and "lung cancer at cumulative internal dose levels lower than 200 millisieverts or 25 centigrays." IOM also concluded that there is limited or suggestive evidence that there is no association between exposure to uranium and "clinically significant renal dysfunction." IOM concluded that there was inadequate evidence to determine whether an association does or does not exist for several other potential long-term health effects.

- Basis for IOM Findings on the potential long-term health effects of DU: IOM states that lung cancer has been the focus of many cohort studies of workers in the uranium processing industry. Many of these studies were large (thousands of subjects) and had a long period of follow-up (more than 20 years). Lung cancer mortality was not increased among workers in most of these cohorts, and IOM focused on the best quality studies in forming its conclusions about radiation exposure and lung cancer. IOM states that the weight of the human evidence indicates little or no clinically important kidney toxicity due to uranium exposure. IOM cited the strongest evidence as the absence of kidney damage in Gulf War veterans exposed to DU from embedded shrapnel. Kidney function was normal in these veterans, years after exposure, despite very high urinary uranium concentrations.

- IOM Recommendations and Research Working Group Response:

1. Continued follow-up of the Baltimore cohort of Gulf War veterans with DU exposure. Long-term studies of the health of other Gulf War veterans at high risk for DU exposure (e.g. cleanup or radiation control units).

    - The RWG concurs with the long-term follow-up of the veterans in the Baltimore cohort, who were injured during friendly fire incidents. This cohort was expanded in 1999, beyond the original 33 individuals. While the Baltimore researchers have seen no definitive evidence of adverse clinical

outcomes associated with uranium exposure to date, the veterans who were involved in the friendly fire incidents will remain under continuing medical surveillance. In addition, since mid-1998, VA and DoD have offered a DU medical evaluation to hundreds of other veterans with potential DU exposure, such as those involved in cleanup operations or radiation control units. To date, the published data have shown that only veterans who have retained metallic fragments have demonstrated persistently elevated urinary uranium levels.

2. Continued follow-up of the cohorts of uranium processing workers.
    - The RWG concurs that the long-term follow-up should continue of cohorts of uranium processing workers. Many of these studies involve employees of manufacturing facilities managed by the Department of Energy or its contractors. Because of the recent increase in interest in the employees of these facilities, ongoing surveillance is likely to intensify in the future. We plan to keep apprised of the results of these studies.
3. Additional studies of the effects of depleted uranium in animals.
    - DoD has funded five toxicology projects that are investigating the health effects of DU in experimental animals (DoD-7A, DoD-7B, DoD-121, DoD-122, DoD-123). In particular, since 1994, the Armed Forces Radiobiology Research Institute (AFRRI) has been investigating the health effects of embedded DU pellets on rats. In Chapter 4, IOM cites the results of several published AFRRI studies. For example, there was no detectable kidney toxicity in rats embedded with DU pellets, even at very high concentrations of urinary uranium. Also, in early 2000, DoD released a Broad Agency Announcement to fund additional studies of health effects of heavy metals in experimental animals, including DU. Outcomes of particular interest include effects on the lung, liver, kidney, and nervous systems; and localized soft tissue responses of embedded fragments. Awards for these projects should occur by late 2000.

Plans for Additional Reviews by the IOM

The present study is only the first phase of a long-term IOM review. VA has already initiated a new contract for the next phase of IOM's review of Gulf War environmental risk factors. The contract calls for the same type of thorough review of peer reviewed literature on health effects from exposure to solvents and pesticides used during the Gulf War. As with the previous study, it will require two years to complete, starting September 1, 2000, at a total cost of $3.57 million. Following that, we anticipate looking at the several other Gulf War risk factors. In addition, the VA and the IOM are committed to issuing updated reports as new evidence appears. VA has not ruled out any exposures as a possible contributor to Gulf War veterans' illnesses.

In summary, a process is in place to review the scientific evidence that becomes available regarding any health consequences from service during the Gulf War and to grant compensation benefits using the same model as was used for Vietnam veterans regarding Agent Orange.

Status Report on Research on Gulf War Veterans' Illnesses

We know that combat casualties do not always result in obvious wounds, and that some veterans from all conflicts return with debilitating health problems. VA recognizes its responsibility for developing effective treatments and prevention strategies for such diseases. Studies clearly show that some Gulf War veterans report a variety of chronic and ill-defined symptoms including fatigue, neurocognitive, and musculoskeletal problems, at rates that are significantly greater than non-deployed veterans.

Four Major Research Initiatives on Illnesses in Gulf War Veterans

Highlights of the ongoing research efforts on Gulf War veterans' illnesses include two major treatment trials, Phase III of the VA National Survey, and a new epidemiological study of amyotrophic lateral sclerosis (ALS) in Gulf War veterans.

As a result of epidemiological findings to date, subgroups of ill Gulf War veterans have been identified for whom trials of potential treatment are appropriate. In the spring

of 1998, the VA Cooperative Studies Program initiated planning for two treatment trials, subsequently known as the "ABT" (antibiotic treatment) and "EBT" (exercise-behavioral therapy) trials. Both trials underwent thorough scientific review and were approved for funding only after rigorous external review provided by the Cooperative Studies Evaluation Committee. Patient characteristics for entry into both trials are similar. All veterans who served in the Gulf between August 1990 and August 1991 are eligible for the studies. Patients are considered to have Gulf War Veterans' Illnesses (GWVI) if they have at least two of three symptoms (fatigue, musculoskeletal pain, neurocognitive dysfunction) that began after August 1990 and that have lasted for more than six months up to the present.

The ABT trial has completed its enrollment of 491 Gulf War veterans at 28 sites throughout the U.S. The study initiated patient accession in May of 1999. The primary hypothesis of the study is that antibiotic treatment directed against mycoplasma species will improve functional status of patients with GWVI who are tested as mycoplasma positive at baseline. The total cost of this treatment trial is approximately $13 million. The trial will be completed in October 2001, when patient follow-up is finished. Preliminary demographic information indicates that 15% of the study participants are women, nearly 20% represent minority groups, 37% have attained an educational level of college or higher, and about 70% are employed. Nearly 85% of patients enrolled in the study exhibit all three symptoms of fatigue, pain, and neurocognitive difficulties.

The EBT trial has completed enrollment of nearly 1,100 Gulf War veterans at 20 sites throughout the U.S. The study initiated patient accessions in April of 1999. The primary hypotheses of the study is that both aerobic exercise and cognitive behavioral therapy (CBT) will significantly improve physical function in veterans with GWVI, and that the combination of CBT and exercise will be more beneficial than either treatment would be alone. The cost of this treatment trial is approximately $9.3 million. The trial will be completed on or about December 2001.

Mr. Chairman, I will now provide you with an update of the VA National Survey of Persian Gulf Veterans authorized by Public Law 103-446.

As you may recall, the National Survey is designed to determine the prevalence of symptoms and illnesses among a national random sampling of Gulf War veterans. The

Survey is being conducted in three phases. Phase I was a population-based mail survey of the health of 30,000 randomly selected veterans from the Gulf War era (15,000 Gulf War veterans and 15,000 non-Gulf War veterans, males and females). The data collection phase is complete and analysis of the data continues. Phase II consisted of a telephone interview of 2,000 non-respondents from Phase I (1,000 from each group) to determine if there are any response differences between respondents and non-respondents. Phase II is complete. In Phase III, 2,000 of the veterans who responded to the postal survey will be invited, along with their family members, to participate in a comprehensive physical examination protocol. These examinations are being conducted at 15 VA medical centers and involve specialized examinations including neurological, rheumatological, psychological, and pulmonological evaluations. When the National Survey is complete we will have a much clearer picture of the prevalence of symptoms and illnesses among Gulf War veterans.

The VA's Office of Research and Development awarded funds for Phase III of the National Health Survey of Persian Gulf Veterans in November 1998. Currently, 15 sites are participating in these physical examinations. Thus far, this study has examined approximately 1,600 veterans, plus 2,000 of their spouses and children. The study will cost approximately $12 million and will complete patient recruitment in May of 2001.

The medical evaluations in Phase III are designed to determine:

- Whether Gulf War veterans have an increased prevalence of the following conditions frequently reported in the literature, compared to a control group of non-deployed veterans: Chronic Fatigue Syndrome (CFS); Fibromyalgia (FM); neurologic abnormalities, including peripheral neuropathy and cognitive dysfunction; and post-traumatic stress disorder (PTSD).
- Whether the specific medical conditions of arthritis, dermatitis, hypertension, bronchitis, and asthma, which have been reported more frequently among Gulf War veterans compared to non-deployed veterans, are at higher prevalence among deployed Gulf War veterans upon objective clinical examination.
- Whether the prevalence of any of these conditions is greater among the spouses of Gulf War veterans than among spouses of non-deployed veterans.

- Whether the prevalence of medical conditions and major birth defects found on a pediatric physical examination in the children conceived after the war is greater for Gulf War veterans than for non-deployed veterans.

Recently, Gulf War veterans have voiced concerns about a possible association between amyotrophic lateral sclerosis (ALS) and service in the war. Although there is no clear indication of an excess rate of ALS among Gulf veterans, the available data could represent an underestimate of the actual rate. Furthermore, preliminary data suggested that the age distribution of cases of ALS in Gulf veterans appeared to be younger than the age distribution of cases of ALS in the general U.S. population. Accordingly, VA is leading a research effort to identify all cases of ALS, or other motor-neuron diseases, occurring among Gulf War veterans. VA is collaborating with DoD, CDC, and various university disease experts to determine the veterans' health status and to describe their exposures to potential causal and risk factors for ALS, based on clinical examinations at VA or non-VA centers of excellence in neurologic diseases. This initial case-finding effort is ongoing, and is planned to continue through February 2001. This study should provide the most definitive information about the rate of ALS among Gulf veterans, and the age distribution of the diagnosed patients.

Other Research Initiatives on Illnesses in Gulf War Veterans

The research program has yielded several important results. Some of the highlights of recent research findings include:

- Population-based epidemiological studies have shown that Gulf War veterans report more symptoms and exposures than non-deployed veterans of the same era.
- The population-based study of Gulf War veterans in Iowa has shown that nearly 90% of Gulf War veterans reported their health status as "good" to "excellent," while the remainder rated their health status as "fair" to "poor," using standard measures of health status. A minority of them (14%) experienced a significant decline in their health status. Declines were noted in physical functioning and social functioning, while mental health scales showed improvement.
- Several major studies suggest that Gulf War veterans do not suffer from a unique, previously-unrecognized syndrome. In particular, four studies have evaluated the

health of thousands of Gulf War veterans who served in: a) the US Air Force; b) the US Navy; c) all three US services; and d) all three services from Great Britain. In each study, Gulf War veterans and comparison groups of non-deployed veterans reported the same patterns of symptoms. The results of these four studies are consistent with IOM's conclusion that "Thus far, there is insufficient evidence to classify veterans' symptoms as a new syndrome." IOM also concluded "All Gulf War veterans do not experience the same array of symptoms. . . Thus, the nature of the symptoms suffered by many Gulf War veterans does not point to an obvious diagnosis, etiology, or standard treatment."

- The RWG has determined that population based longitudinal studies to determine the long-term health of Gulf War veterans are a high priority. There are two population based longitudinal studies underway that are supported by DoD and the Centers for Disease Prevention and Control (CDC). They are Iowa (CDC and DoD), and the United Kingdom (U.S. DoD). Altogether, these two studies are following up a total of approximately 12,000 veterans. Each of these studies has used questionnaires, including physical symptoms, psychological symptoms, and exposures during the Gulf War. Both the Iowa and United Kingdom studies have included comprehensive medical histories and physical examinations. VA will request proposals to conduct a pilot of a longitudinal study based on its National Gulf War Survey.
- Neurobehavioral studies of Gulf War veterans and control populations suggest that some Gulf War veterans may have brain function abnormalities in such areas as memory, cognition, and motor control. The current RWG research portfolio includes seven studies using methods of sophisticated brain imaging such as conventional and functional magnetic resonance imaging (fMRI), and magnetic resonance spectroscopy.
- VA has developed a plan to establish two new Centers for the Study of War-Related Illnesses. These new Centers will assist VA in the development of appropriate preventive strategies to minimize illness and injury following future conflicts, including both combat and peace-keeping operations, and to develop new approaches for improving the care of active-duty and veteran patients with war-related illnesses.

VA has released its Request for Proposal for these new Centers and plans to fund them within the next few months.

- In early 2000, DoD published Broad Agency Announcements to announce the availability of research funding on four topics. The selection and awarding of funds will be completed by the end of 2000. The topics are:
    1. Toxicity of heavy metals that are relevant to the military, including DU;
    2. Biomarkers to assess toxic chemical exposures and health effects;
    3. Consequences of deployment stress on health and performance; and
    4. Physiologically based methods to assess health consequences of deployment.

Conclusions

As the federal research program continues to provide more results, we will substantially increase our understanding of Gulf War veterans' illnesses, which, in turn, will enhance our ability to diagnose and treat them. In addition, this newly gained knowledge will enhance prevention and intervention in illnesses in participants of future deployments.

Mr. Chairman, thank you again for permitting me this opportunity to summarize our work to date so that, using science, we may better understand the health problems of Gulf War veterans. You have my assurance that we will continue this effort to resolve or ameliorate health problems in this population to the greatest extent possible.

Mr. Chairman, I will conclude my testimony here and am happy to answer any questions you or other Committee members may have.

Mr. SHAYS. Already covered, but it doesn't hurt to ask. Let me just, before recognizing Mr. Sanders, say they wish a lot of these studies had begun 10 years ago. I think that many of them are very important and valuable. I think that it's good they're happening. I wish they could have happened sooner, but I guess we call that progress, and Mr. Sanders.

Mr. SANDERS. Thank you very much, Mr. Chairman. As you know, I have been very critical of the DOD and the VA for many years in this area, but I do want to single out Jack Feussner as somebody who I think for many, many years has been trying to do the right thing, Jack and I appreciate the work you have done.

Let me just ask you, you remember, Dr. Feussner, a couple of years ago at a hearing, I had indicated to you that I was distressed that there was some apparently breakthrough work being done around the country, and I asked you if the VA had begun the process of trying to replicate some of that work, tell us whether it was right or wrong, and I think out of that discussion with Chairman Shays' help and so forth, you began a clinical trial based on I think the work of Dr. Nicholson in California dealing with doxycycline, and I know that clinical trial is going on in a hospital in White River Junction in Vermont, hospitals all over this country, and the thesis was that large doses of doxycycline over a long period than had previously been given seemed to indicate that there would be some alleviation of symptoms.

That was Nicholson's hypothesis. You were testing it. Do you have anything to report to us today about the progress of that study?

Dr. FEUSSNER. Yes, sir, I have progress to report. You're quite correct, the study continues. You're also quite correct to assert that the treatment was doxycycline and the duration of the doxycycline was quite long, 1 year. Because this is a—while tetracycline is not an experimental or novel therapy, the use of tetracycline——

Mr. SANDERS. Doxycycline is what we're talking about.

Dr. FEUSSNER. Yes, sir.

Mr. SANDERS. Tetracycline is the same?

Dr. FEUSSNER. Doxycycline is a specific brand of tetracycline. I will try to keep it straight. At any rate, this trial, as you recall, was planned as a collaborative effort between VA and DOD, went through a very rigorous scientific review process that actually included a formal request for an FDA IND, an investigational new drug not because the drug is investigational, but because the condition for which the drug is being used is not approved by the FDA.

We initiated the trial formally in May 1999. The goal was to study—enroll up to 450 Gulf war veterans at 28 sites throughout the United States. We have achieved that goal. As a matter of fact, as we intended to close enrollment in the trial, we had a number of veterans who wished to participate, despite the fact that we had met our patient sample size quota. Nonetheless, we included an additional 41 veterans into the trial. The total sample size now is 491. The patient recruitment period is done and the patients are currently in the process of going through that 1-year treatment.

Mr. SANDERS. Does that include, that 491, is that some of those—half of those people are getting placebos?

Dr. FEUSSNER. Correct, yes, approximately 50/50.

So the patients are all enrolled in the trial and are all now being treated with the active agent doxycycline, or placebo, are in the process of being followed on that treatment over the course of 1 year. I expect the study to be complete, the followup to be complete next summer, approximately June or so, and that we will have the final result some time after that, some time probably within the next 90 days of completion of the trial. So the trial has been a success.

Mr. SANDERS. In the sense of organizing it?

Dr. FEUSSNER. In the sense of organizing, recruiting patients, but I can't tell you what the results are yet.

Mr. SANDERS. So in June you will be beginning the process—you'll be completing the study and beginning the process of analyzing the results?

Dr. FEUSSNER. That is correct. You may recall, Congressman, we also started another major trial, that one much more difficult. We call it exercise and behavioral therapy, organizationally more complex for treatment groups. Similarly, we have closed the patient recruitment for the EBT trial. There are four treatment groups, usual care, exercise only, cognitive behavioral therapy only or both interventions. We did not quite meet our goal for patient inclusion. We'd hope to have approximately 1,300 patients enrolled. We have succeeded, however, in enrolling 1,100 patients in the trial and the trial, whatever the result, will be statistically robust. So while we had hoped to have a few more patients, we are very gratified that 1,100 Gulf war veterans have volunteered to help us with the trial. That trial, as you recall, is a little later in the process than the ABT. I don't expect the final end point of that trial until the fall of 2001, and probably around September or so with the same issue that at that point, we will begin the analysis and should have the results—pretty good result within a 90-day period.

Mr. SANDERS. And I presume—is my time up, Mr. Chairman?

Mr. SHAYS. No, no.

Mr. SANDERS. I presume that if one or both of those studies indicate that approach alleviates symptoms—that approach will become recommended form of treatment throughout the VA system.

Dr. FEUSSNER. Yes, I would say the answer to that question would be yes, that the trials, as you know, the trials are large, they're very expensive and they are constructed to be definitive. So that if the result is positive, then the treatment is known to work, and if the result is negative, then the treatment is known not to work.

Mr. SANDERS. Dr. Feussner, I am, as you know, not a scientist, and the way my mind works, as I mentioned to you before, and I appreciate you moving with that type of approach, is that if somebody is doing interesting work, we test the hypothesis, and frankly, this work was based on what Nicholson had indicated out in California, is that correct? More or less through other people?

Dr. FEUSSNER. As you recall, sir, Dr. Nicholson's work was quite controversial.

Mr. SANDERS. I sure do.

Dr. FEUSSNER. There were two observations. While his results were controversial, one of our own physicians Dr. Gordon had anecdotal experience——

Mr. SANDERS. That's right.

Dr. FEUSSNER [continuing]. On his own in a significant number, not two or three, but perhaps several dozens of patients where he had observed clinically that he had tried the therapy and believed that the therapy worked.

Mr. SANDERS. Dr. Gordon from Manchester, New Hampshire?

Dr. FEUSSNER. Yes, sir.

Mr. SANDERS. That's right. And it seems to me that a good administrator, such as yourself, listens to those people, who may only have anecdotal evidence of some success. OK. So I am applauding you for this, but let me ask you this, getting back to the question I asked Dr. Sox a moment ago, if there appears to be some breakthroughs, what you're saying is if Dr. Gordon came to you and said listen, I'm applying this treatment, it appears to be working, let's go further with it and you said yeah, let's go further with it, Nicholson did his work, and I think you did exactly the right thing, what about the work that people like Hayley or Urnovitz or Miller are doing out there? There is also anecdotal evidence that there may be some breakthroughs. Are you prepared to say come on in, let's work together, let's see, in fact, to answer the question that Dr. Sox raised with Hayley's work that the sampling was too small, there hasn't been enough replication, are you going to help us—tell us whether or not Hayley is on to something or whether he's not?

Dr. FEUSSNER. Well, before we get to Dr. Hayley's work specifically, Congressman Sanders, you will recall that some years ago, I believe in 1998, that VA announced an open-ended what we call RFP, request for proposals, DOD calls BAA, broad area announcement, indicating our receptivity to treatment trials of any novel therapy agent. That RFP is still active, but I will concede that we perhaps should reannounce it just to make sure that those that need to know are reminded that that is still active.

Mr. SANDERS. What I am asking, Dr. Feussner, you know what I'm asking, are we welcoming in the door people who have controversial ideas who are not quote unquote, peer reviewed by folks who have not given us any information in 10 years? Are you having the courage to go out and say, look, people may—I may be attacked for going to somebody who is controversial, but I'd rather be attacked for going to somebody who is controversial and may contribute something to our knowledge rather than go back to the same old folks who 20 years from now tell us we don't know the cause. Are you prepared to do that, to take the heat?

Mr. SHAYS. You recognize that's a loaded question, don't you?

Mr. SANDERS. You understand where I am coming from?

Dr. FEUSSNER. Sir, I certainly do understand where you're coming from, and what I would say is I think our actions do speak to that issue, and that is, that we have followed up with larger scale research, looking at reasonable testable hypotheses, specifically with regard to Dr. Hayley. Dr. Hayley published preliminary work in the Journal of the American Medical Association exploring possible definition for a number of Gulf war syndromes. You will recall that very early on after that work is published, I had the opportunity to testify before the committee. I think that Dr. Sox's point is well taken. Dr. Hayley studied a small number of study subjects.

His response rate in the initial study, even in a highly selected patient population, was only 40 percent. There were no controls.

But the observation bore attention, OK. I mean, he put something on the table. Now, the follow-on to that is it's—as you know, because we've talked about this a lot, scientific process. It's not important for the initial investigator to replicate his or her own work but for other scientists to do that.

We have supported four follow-on studies looking at those syndromes, three in the United States and one in the United Kingdom. The United Kingdom was published in the Journal Lancet by Dr. Wesley. We have the Naval Health Research Center in San Diego. We have the CDC study of Fukuda and colleagues, and we have the Iowa study just recently published this year in the American Journal of Medicine. None of those studies is able to replicate Dr. Hayley's initial observations in terms of finding the kinds of unique syndromes that Dr. Hayley found in his preliminary hypothesis-generating research.

What we are left with in that effort is, one, we have followed on the effort to replicate the work. We have not been able to replicate the work at this point in time. But actually, there is yet another study that we are supporting in collaboration with researchers at GW using the same analytical strategy, etc.

Mr. SANDERS. What you're saying is you've taken Hayley's work seriously, you're putting money and resources into trying to replicate it, at this point that has not happened.

Dr. FEUSSNER. In that particular one we have not been able to replicate the work.

With regard to the work on the structural brain disease, we have talked about that at the hearing in February, and we have a number of studies ongoing that are looking also at structural brain disease. The most—and so, an effort is underway to try to explain, replicate, extend that observation. The most recent observation, actually I haven't had an opportunity to go over in detail myself. It is quite recent, within the last week or so, looking at neurotransmitters, chemicals in the brain that tell other parts of the brain what to do, and since the brain tells the rest of the body everything to do, very important, called—dopamine is the chemical.

We haven't taken a hard look at that yet, but what I will tell you, the worry here has to do with Parkinson's disease, and independent of this issue with Hayley, VA is currently reviewing, as a result of another RFP VA is currently reviewing, and hopefully later this calendar year will fund up to six major centers, research centers devoted to the study of Parkinson's disease and movement disorders. We call them PADRECC's, Parkinson's Disease, Research Education and Clinical Centers, modelled after the VA-funded geriatric centers. So that we will have the capacity, I believe, within—at least within VA, certainly within the broader scientific community, to follow on those observations.

So I think what I'm doing, Congressman, is giving you a long answer to a short question.

Mr. SANDERS. It's a good answer. Let me ask you this and I'll give the mic back to the chairman. I remember, sometimes there are instances where things occur and you never forget them, but I remember meeting with many Vermont veterans who are suffer-

ing from Gulf war illness, and one of the symptoms, many of them relayed to me is when they were exposed to perfume or detergent smells or other chemical presence, gasoline fuels, they would become sick, which suggested to me that we're looking perhaps at what might be called multiple chemical sensitivity, and as you know, I am sympathetic to the work that Dr. Claudia Miller and others are doing. Can you tell us a little bit about some of the research the VA may or may not be doing in following up on the issue of multiple chemical sensitivity in Gulf war illness?

Dr. FEUSSNER. I think what I would have to say, Congressman Sanders, is that the last time you asked that question, I don't have much of a different answer to give you this time. We have about half a dozen or so research projects looking at the issue of multiple chemical sensitivity. They're currently active. In a response to a meeting that we had with you in your chambers some time ago, we invested a considerable amount of energy trying to forge a collaboration between Dr. Miller and VA investigators, both in San Antonio and, as I recall, in Tucson with Dr. Iris Bell, who's testified before you in the past.

We have also indicated, as you know, the interest in explicitly looking at prospective treatment trials and also, as you know, some of the difficulty in pursuing those ideas aggressively relate to the infrastructure that is required in order to do the research. It's not as——

Mr. SANDERS. Let me just jump in and bring this to the point. To the best of my knowledge the U.S. Government, despite the widespread feeling of many physicians, certainly not all, that multiple chemical sensitivity is a serious disease not only facing Gulf war veterans but the American population. Correct me if I'm wrong, Dr. Feussner, but I don't know that the Veterans Administration or DOD owns what is called an environmental chamber where we can do scientific studies regarding treatment of multiple chemical sensitivity. Is that a fair statement? I know we're trying to get funding for it, but it's beyond my comprehension that the U.S. Government doesn't own one of those units quite yet.

Dr. FEUSSNER. I think I answered that question the last time and said yes, the U.S. Government does own these facilities. I am searching my hard drive to find those data, sir. What I can tell you is the VA does not. I can't recollect about DOD. I do recollect that EPA has such laboratories in the research triangle in North Carolina, and I do believe that DOD has several of these facilities, but I cannot remember the last time I looked this up. I'll have to——

Mr. SANDERS. Short term memory loss, multiple chemical sensitivity, there it is. Thank you very much, Mr. Chairman.

Mr. SHAYS. I'm just going to have a slight advertisement for a committee meeting that we're having next week on Gulf war illnesses. The Royal British legion formed a Gulf veterans group some years ago to provide a focus for Gulf veterans issues. It is made up of Gulf veterans, parliamentarians, representatives of VSOs and service welfare organizations and medical and scientific advisers. A delegation from the Gulf war veterans group visited the United States in July 1995 and similar group intend to visit Washington, DC, from October 2nd to 6th. We will be meeting with a group on Wednesday, October 4th from 10 a.m. in room 2154 with

Lord Morris, the distinguished parliamentarian, with a background in trades and union members; Colonel Terry English, director of welfare at the Royal British legion; Kathy Walker, director of welfare, the Soldiers, Sailors and the Airmen Families Association; Dr. Norman Jones, medical adviser, Royal British Legion; Mr. John Nichol, author, Gulf war veteran and ex-POW; Professor Malcolm Hooper, scientific adviser, Gulf Veterans Association.

Let me first ask you, Dr. Brown, is there anything that you would want to respond to Mr. Sanders, any comment or observation?

Dr. BROWN. No. When it comes to research issues, Jack is your man.

Mr. SHAYS. OK. Well, I'll ask either one of you, how many of the 83 research projects—there are 192 research projects in Gulf war veterans illnesses at various stages of completion, 83 have been completed, and I want to know of the 83 projects completed, how many have been published and peer reviewed?

Dr. FEUSSNER. I'll have to get that information for you, Congressman Shays.

Mr. SHAYS. Could someone else give us that?

Dr. FEUSSNER. We don't have that off the top of our heads.

Mr. SHAYS. How many completed projects involve sarin and have been published and peer reviewed?

Dr. FEUSSNER. I will have to get those data for you as well.

Mr. SHAYS. How many involving PB?

Dr. FEUSSNER. How many are already finished and published?

Mr. SHAYS. Published and peer reviewed.

Dr. FEUSSNER. I think it's approximately six to eight.

Mr. SHAYS. OK. How many as relates to DU, depleted uranium?

Dr. FEUSSNER. I would say, again, probably six or seven.

Mr. SHAYS. And how many involving vaccines?

Dr. FEUSSNER. I don't know the answer to that question. I'll have to get you those data.

Mr. SHAYS. I know you will do that. I do want to ask the questions though. What yet unpublished studies are underway which would address the long-term effects of exposure to these toxic agents?

Dr. FEUSSNER. Well, there are quite a large number of projects that are still ongoing. For example, in PB, the total number of funded projects is about 30. With regard to chemical weapons, there are about 22. The DU focus at the moment in humans is pretty much limited to followup of the friendly fire soldiers in Baltimore, and there are a small number of probably four or five animal studies in DU.

Mr. SHAYS. OK.

Dr. FEUSSNER. Did I answer all the parts?

Mr. SHAYS. Well, it's a pretty extensive question. You said there are many. Do you think, in fact, there are many?

Dr. FEUSSNER. Yes.

Mr. SHAYS. OK. And by "many," you would give a number of what approximately?

Dr. FEUSSNER. For which issue?

Mr. SHAYS. I just asked what yet unpublished studies are underway which address the long-term effects of exposure to these toxic agents which involve those four agents.

Dr. FEUSSNER. Yes, I could do the math real quick.

Mr. SHAYS. Some you don't know. You said many. Are we talking 20, are we talking 80? I mean, what are we talking about?

Dr. FEUSSNER. In terms of total number of projects I think we're talking about in the ballpark of perhaps 100.

Mr. SHAYS. And you will get back to us and document those?

Dr. FEUSSNER. You not only want the number of projects, you want the number of projects, those finished and the publication?

Mr. SHAYS. Right.

Dr. FEUSSNER. Yes, sir, I'll have to get you that data.

[The information referred to follows:]

INSERT

Questions For The Record
Concerning the September 27, 2000, Hearing

for
Dr. John R. Feussner
Chief Research and Development Officer
Veterans Health Administration, Department of Veterans Affairs

from
The Honorable Christopher Shays
Chairman,
Subcommittee on National Security, Veterans Affairs & International Relations
U. S. House of Representatives

Question: How many of the 83 research projects completed have been published and peer-reviewed?

Answer: Fifty (50) of the 83 completed projects have produced peer-reviewed publications. Thirty-three (33) projects have not produced peer-reviewed publications yet.

Question: How many completed projects involving sarin have been published and peer-reviewed? Where? Who? What yet unpublished studies are underway which will address the long-term effects of exposures to sarin?

Answer: There are nine (9) projects evaluating the long-term effects of sarin exposure in experimental animals. Three (3) of these projects are complete, which have produced two (2) peer-reviewed publications, listed below. Six (6) projects are ongoing.

Olson, CT, Blank, JA, and Menton, RG. Neuromuscular effects of low-level exposures to sarin, pyridostigmine, DEET, and chlorpyrifos. *Drug and Chemical Toxicology* 1998; 21 Suppl 1:149-169. (Project DoD-54)

Khan, WA, Dechkovskaia, AM, Herrick, EA, Jones, KH, and Abou-Donia, MB. Acute sarin exposure causes differential regulation of choline acetyltransferase, acetylcholinesterase, and acetylcholine receptors. *Toxicological Sciences* 2000; 57(1):112-120. (Project DoD-72)

There are also three (3) projects evaluating the effects on Gulf War veterans who may have been exposed to low-levels of sarin due to the demolitions at Khamisiyah, Iraq in March 1991. One of these projects is complete and it has

produced one peer-reviewed publication, listed below. Two (2) projects are ongoing.

Gray, GC, Smith, TC, Knoke, JD, and Heller, JM. The postwar hospitalization experience of Gulf War veterans possibly exposed to chemical munitions destruction at Khamisiyah, Iraq. *American Journal of Epidemiology* 1999; 150(5):532-540. (Project DoD-1B)

Question: How many completed projects involving PB have been published and peer-reviewed? Where? Who? What yet unpublished studies are underway which will address the long-term effects of exposures to PB?

Answer: There are thirty-one (31) projects evaluating the effects of pyridostigmine bromide (PB). Sixteen (16) of these projects are complete, which have produced fourteen (14) peer-reviewed publications, listed below. Fifteen (15) projects are ongoing.

Kaiser, KS, Hawksworth AW, and Gray GC. Pyridostigmine bromide intake during the Persian Gulf War is not associated with handgrip strength. *Military Medicine* 2000; 165(3):165-168. (Project DoD-1A)

McCain, WC, Lee, R, Johnson, MS, Whaley, JE, Ferguson, JW, Beall, P, and Leach, G. Acute oral toxicity study of pyridostigmine bromide, permethrin, and DEET in the laboratory rat. *Journal of Toxicology and Environmental Health* 1997; 50(2):113-124. (Project DoD-10)

Marino MT, Schuster, BG, Brueckner, RP, Lin, E, Kaminskis, A, and Lasseter, KC. Population pharmacokinetics and pharmacodynamics of pyridostigmine bromide for prophylaxis against nerve agents in humans. *Journal of Clinical Pharmacology* 1998; 38(3):227-235. (Project DoD-11)

Van Haaren, F, De Jongh, R, Hoy, JB, Karlix, JL, Schmidt, CJ, Tebbett, IR, and Wielbo, D. The effects of acute and repeated pyridostigmine bromide administration on response acquisition with immediate and delayed reinforcement. *Pharmacology, Biochemistry, and Behavior* 1999; 62(2):389-394. (Project DoD-37)

Hoy, JB, Cody, BA, Karlix, JL, Schmidt, CJ, Tebbett, IR, Toffollo, S, Van Haaren, F, and Wielbo, D. Pyridostigmine bromide alters locomotion and thigmotaxis of rats: gender effects. *Pharmacology, Biochemistry, and Behavior* 1999; 63(3):401-406. (Project DoD-37)

Hoy, JB, Cornell, JA, Karlix, JL, Schmidt, CJ, Tebbett, IR, and van Haaren, F. Interactions of pyridostigmine bromide, DEET, and permethrin alter locomotor behavior of rats. *Veterinary and Human Toxicology* 2000; 42(2):65-71. (Project DoD-37)

Hoy, JB, Cornell, JA, Karlix, JL, Tebbett, IR, and van Haaren, F. Repeated coadministrations of pyridostigmine bromide, DEET, and permethrin alter locomotor behavior of rats. *Veterinary and Human Toxicology* 2000; 42(2):72-76. (Project DoD-37)

Van Haaren, F, Cody, B, Hoy, JB, Karlix, JL, Schmidt, CJ, Tebbett, IR, and Wielbo, D. The effects of pyridostigmine bromide and permethrin, alone and in combination, on response acquisition in male and female rats. *Pharmacology, Biochemistry, and Behavior* 2000; 66(4):739-746. (Project DoD-37)

Olson, CT, Blank, JA, and Menton, RG. Neuromuscular effects of low-level exposures to sarin, pyridostigmine, DEET, and chlorpyrifos. *Drug and Chemical Toxicology* 1998; 21 Suppl 1:149-169. (Project DoD-54)

Somani, SM, Husain, K, Asha, T, and Helfert, R. Interactive and delayed effects of pyridostigmine and physical stress on biochemical and histological changes in peripheral tissues of mice. *Journal of Applied Toxicology* 2000; 20(4):327-334. (Project DoD-62)

Sinton, CM, Fitch, TE, Petty, F, and Haley, RW. Stressful manipulations that elevate corticosterone reduce blood-brain barrier permeability to pyridostigmine in the rat. *Toxicology and Applied Pharmacology* 2000; 165(1):99-105. (Project DoD-65)

Servatius RJ, Ottenweller, JE, Beldowicz, D, Guo, W, Zhu, G, and Natelson, BH. Persistently exaggerated startle responses in rats treated with pyridostigmine bromide. *Journal of Pharmacology and Experimental Therapeutics* 1998; 287(3):1020-1028. (Project VA-5D)

Servatius, RJ, Ottenweller, JE, Guo, W, Beldowicz, D, Guanping, Z, and Natelson, BH. Effects of inescapable stress and treatment with pyridostigmine bromide on plasma butyrylcholinesterase and the acoustic startle response in rats. *Physiology and Behavior* 2000; 69(3):239-246. (Project VA-5D)

Drake-Baumann, R, and Seil, FJ. Effects of exposure to low-dose pyridostigmine on neuromuscular junctions in vitro. *Muscle and Nerve* 1999; 22(6):696-703. (Project VA-6C)

Question: How many completed projects involving DU have been published and peer-reviewed? Where? Who? What yet unpublished studies are underway which will address the long-term effects of exposures to DU?

Answer: There are five (5) projects evaluating the effects of depleted uranium (DU). One (1) of these projects is complete, which has produced three (3) peer-reviewed publications, listed below. Four (4) projects are ongoing.

Miller, AC, Fuciarelli, AF, Jackson, WE, Ejnik, EJ, Emond, C, Strocko, S, Hogan, J, Page, N, and Pellmar, T. Urinary and serum mutagenicity studies with rats implanted with depleted uranium or tantalum pellets. *Mutagenesis* 1998; 13(6):643-648. (Project DoD-7A)

Pellmar, TC, Fuciarelli, AF, Ejnik, JW, Hamilton, M, Hogan, J, Strocko, S, Emond, C, Mottaz, HM, and Landauer, MR. Distribution of uranium in rats implanted with depleted uranium pellets. *Toxicological Sciences* 1999; 49(1):29-39. (Project DoD-7A)

Pellmar, TC, Keyser, DO, Emery, C, and Hogan, JB. Electrophysiological changes in hippocampal slices isolated from rats embedded with depleted uranium fragments. *Neurotoxicology* 1999; 20(5);785-792. (Project DoD-7A)

Question: How many completed projects involving vaccines have been published and peer-reviewed? Where? Who? What yet unpublished studies are underway which will address the long-term effects of exposures to vaccines?

Answer: There are three (3) projects evaluating the effects of vaccines, none of which is complete. Two (2) of these ongoing projects have produced peer-reviewed publications, listed below. (Note that there are many published studies on vaccines that did not arise from the research portfolio focusing on Gulf War illnesses.)

Fukuda, K, Nisenbaum, R, Stewart, G, Thompson, WW, Robin, L, Washko, RM, Noah, DL, Barrett, DH, Randall, B, Herwaldt, BL, Mawle, AC, and Reeves, WC. Chronic multisymptom illness affecting Air Force veterans of the Gulf War. *Journal of the American Medical Association* 1998; 280(11):981-8. (Project HHS-2)

Hotopf, M, David, A, Hull, L, Ismail, K, Unwin, C, and Wessely, S. Role of vaccinations as risk factors for ill health in veterans of the Gulf War: a cross sectional study. *British Medical Journal* 2000; 320:1363-1367. (Project DoD-39)

Mr. SHAYS. I understand. According to a January 2000 General Accounting Office report on Gulf war illnesses, the Department of Veterans Affairs stated that the research working group, which I'll refer to as RWG, would, "establish a date in fiscal year 1999 or fiscal year 2000 for publishing its assessment of progress toward addressing the 21 research objectives that's identified in 1995." When will the research group assessment's of progress toward addressing the 21 research objectives be published?

Dr. FEUSSNER. We've actually made a substantial progress in this area, Congressman. We discussed this at our last hearing, and the majority, 11 of the 15 papers that we had commissioned at that time are in draft form. We have worked with a very prestigious medical journal, and the editor of that journal to not only produce these papers for the Congress, but to produce these papers for the larger community. We have a commitment from——

Mr. SHAYS. Isn't that the key? The larger community is complaining to us that they're not getting access to this research.

Dr. FEUSSNER. Yes, I think that is the issue.

Mr. SHAYS. To date, we don't really have any published.

Dr. FEUSSNER. The papers have received preliminary review by the editor of the journal already, but the next step for the manuscripts will be to go out to independent experts to get an additional episode of review. I would hope that the manuscripts would be published electronically after the 1st of the year, perhaps the second quarter of fiscal year 2001. We have discussed with the editor the possibility of publishing the manuscripts electronically while we await for the manuscripts to appear in print. It is my hope that we can have the manuscripts in electronic format between January and March and in print as a special supplement, probably between March and May.

Mr. SHAYS. So basically you're in fiscal year——

Dr. FEUSSNER. I am in fiscal year 2001.

Mr. SHAYS. Right. You're really at the end of that fiscal year—well, it starts in September.

Dr. FEUSSNER. Yes.

Mr. SHAYS. Not in the end. You're kind of in the middle. What is the research working group's role with the Military and Veterans Health Coordinating Board?

Dr. FEUSSNER. Well, the research working group, the Military and Veterans Health Coordinating Board has three subcomponents underneath the executive, the executive leader. The research is one of those three subgroups. Within the research group, there will really be two primary foci. The first will be the Gulf war research activities, since 60 percent of these projects are incomplete. As a matter of fact, I think just in fiscal year 1999 and 2000, we have launched 42 additional studies.

The second component of the research activity within the military and veterans coordinating board will deal more specifically with the generic issue of post deployment health and three major, at least three major interests within that area will include an effort to improve the situation with regards to systematically obtaining baseline data so that after subsequent deployments, we will systematically have baseline data; systematically collect data through time on the soldiers which would also require an integration and

a merging of the VA and DOD data bases; and then increasingly apply research activities or research results became available that could document exposures.

Mr. SHAYS. To what extent will the absorption of the RWG into the new Military and Veterans' Health Coordinating Board diminish the RWG's focus on Gulf war illnesses, veterans illness research?

Dr. FEUSSNER. Well, it is my intent that it not diminish the focus on Gulf war veterans' illness, and given the incomplete status of the formal research and the emerging research that is going to be initiated with regards to post deployment health issues, I would imagine over the next period of time, say the next 3 or 4 years, that the dominant research effort within that larger group will continue to be Gulf war research projects.

Mr. SHAYS. I'm going to try to finish because Mr. Sanders and I need to vote, but to what extent is the new board fully operational?

Dr. FEUSSNER. The new board has already engaged in a series of meetings several weeks ago. All leaders of the boards and a larger community of involved participants had a 2-day retreat at Andrews Air Force Base. We are completing the formal strategic planning process for the coordinating board and have identified the three leaders of the three major subgroups.

Mr. SHAYS. So you haven't started being operational yet but you're at that point?

Dr. FEUSSNER. I think that's fair.

Mr. SHAYS. According to a General Accounting Office, GAO, January 2000 report on Gulf war illnesses, questions remain regarding, "how many veterans have unexplained symptoms and whether those who have received care in VA facilities are getting better or worse." What progress has been made toward developing a system of tracking clinical efforts and treatment outcomes among sick Gulf war veterans?

Dr. BROWN. I'll take a stab at that. We have a number of ways in which we track the health of Gulf war veterans. The Institute of Medicine recently released a report that I'm sure you're aware of which made the point that if we really want to study the long-term health consequences of service in the Gulf war, that is, your question whether veterans are getting better or worse are staying the same, that you need to set up appropriate longitudinal studies to follow those populations.

We have a couple of studies already underway that are looking at subgroups of veterans. Dr. Feussner mentioned the Iowa study. I also want to make this committee aware, we just published a report just last April on a study that was looking at the health of all Gulf war veterans, called National Veterans Health Survey, looked at the health of all Gulf war veterans across the board. I can provide the committee with a copy of the report. It found similarly to other studies that when you look at a national survey of all Gulf war veterans, that you find greater rates of symptoms, greater rates of illnesses in terms of self-reported symptoms, and a number of other findings. It is unique in that it's the only study that looks across the board at all veterans, and it's our intention—it's my office's intention to follow that study up in a longitudinal sense.

Mr. SHAYS. Basically though what I am hearing you say, we really don't have a system yet to track.

Dr. BROWN. I think we do have some initial data.

Mr. SHAYS. You have data but you don't have a system, you are not tracking all these.

Dr. BROWN. The system that would do that for us would be a longitudinal study.

Mr. SHAYS. "Would be" is not——

Dr. BROWN. We don't have that in place yet.

Mr. SHAYS. This is all. And finally, what is the Department of Veterans Affairs doing about obtaining access to classified information? This really galls me that we don't have information. I mean we had the DOD who said our troops weren't exposed to offensive chemical exposure, and yet they were exposed to defensive chemical exposure. So I want to know what the VA's doing. Are we just lying back or are we trying to get this information?

Dr. FEUSSNER. In the research mode, we have not made efforts to get classified information. Two comments. The first is that my understanding is that the IOM will gain access at least to unpublished information about anthrax research in a new study that is being undertaken by them, and that with regard to CW, chemical weapons, issues that both the Presidential Advisory Commission and the Senate Veterans Affairs Investigating Committee had access to that classified information.

Mr. SHAYS. The challenge we do have is the IOM did not have access to certain information.

Dr. FEUSSNER. That is correct.

Mr. SHAYS. And I think it galls both me and Congressman Sanders that that's not made available, and it would strike me that anybody who's worked with our veterans would demand the same, so I just plead with you to be a little more aggressive. We will. We'd like you to be as well. I think what we'll do, I usually invite comments, if you have a 30 second comment either one of you, I'd welcome that, but we need to get voting. Any comment?

Dr. FEUSSNER. No, sir.

Mr. SHAYS. Thank you both for being here.

[Whereupon, at 12:05 p.m., the subcommittee was adjourned.]

[Additional information submitted for the hearing record follows:]

JACK METCALF
2ND DISTRICT, WASHINGTON
COMMITTEE ON
TRANSPORTATION
AND INFRASTRUCTURE

Congress of the United States
House of Representatives
Washington, DC 20515–4702

COMMITTEE ON BANKING
AND FINANCIAL SERVICES

CHAIR, REPUBLICAN HOUSING
OPPORTUNITY CAUCUS

August 29, 1997

Mr. James F. Hinchman
Acting Comptroller General
U.S. General Accounting Office
441 G. Street NW
Washington DC 20548

Dear Mr. Hinchman:

My office has been contacted by several veterans and other constituents concerned about recent reports that the presence of antibodies for synthetic squalene has been discovered in blood samples of some Gulf War veterans.

I would like to request that you do a preliminary investigation into these reports. It is important that Members of Congress be fully informed of the facts surrounding this issue. If I can be of further assistance, please feel free to contact either myself, or Norma Smith in my Everett office.

Thank you for your attention to this matter.

Sincerely,

Jack Metcalf

WASHINGTON OFFICE:
1510 LONGWORTH HOB
WASHINGTON, DC 20515
(202) 225-2605

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2930 WETMORE AVENUE, #901
EVERETT, WA 98201
(206) 252-3188
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BELLINGHAM OFFICE:
322 NO. COMMERCIAL, #203
BELLINGHAM, WA 98225
(360) 733-4500

GAO
United States
General Accounting Office
Washington, D.C. 20548

National Security and
International Affairs Division

November 14, 1997

The Honorable Jack Metcalf
House of Representatives

Dear Representative Metcalf:

This letter confirms our intent to provide you with information pursuant to your August 19, 1997 request that we conduct a preliminary investigation in reports that antibodies for synthetic squalene have been discovered in blood samples of some Gulf War veterans. Norma Smith of your Everett, Washington office provided us with background regarding your request in conversations on August 29 and September 9, 1997.

Due to the complexity of issues addressed in you August 29 letter, we need to proceed with a separate design phase to examine what preliminary evidence exists for these allegations. The objectives of the study will address the following issues:

- Has DOD ever performed or sponsored any research on synthetic or natural squalene or squalane;
- was synthetic squalene used as an adjuvant in any developmental drugs and/or vaccines;
- are there any pharmaceutical firms involved in the development and production of drugs using squalene in any form;
- what tests have been done regarding its safety, efficacy and effectiveness;
- have our troops or DOD civilian personnel ever been given squalene in any form. If yes, for what purpose and under what circumstances?

The design phase will be completed by January 15, 1998. We will remain in contact with your staff, and by the end of January, we will provide you with a projected completion date for the total study. If you have any questions regarding this work, please contact me at (202) 512-3092, or my Assistant Director, Sushil Sharma, at (202) 512-3460.

Sincerely yours,

Kwai-Cheung Chan
Director
Special Studies and Evaluation

Experimental and Molecular Pathology 68, 55–64 (2000)
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Antibodies to Squalene in Gulf War Syndrome

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Received September 23, 1999

Gulf War Syndrome (GWS) is a multisystemic illness afflicting many Gulf War-era veterans. The molecular pathological basis for GWS has not been established. We sought to determine whether the presence of antibodies to squalene correlates with the presence of signs and symptoms of GWS. Participants in this blinded cohort study were individuals immunized for service in Desert Shield/Desert Storm during 1990–1991. They included 144 Gulf War-era veterans or military employees (58 in the blinded study), 48 blood donors, 40 systemic lupus erythematosus patients, 34 silicone breast implant recipients, and 30 chronic fatigue syndrome patients. Serum antibodies to squalene were measured. In our small cohort, the substantial majority (95%) of overtly ill deployed GWS patients had antibodies to squalene. All (100%) GWS patients immunized for service in Desert Shield/Desert Storm who did not deploy, but had the same signs and symptoms as those who did deploy, had antibodies to squalene. In contrast, none (0%) of the deployed Persian Gulf veterans not showing signs and symptoms of GWS have antibodies to squalene. Neither patients with idiopathic autoimmune disease nor healthy controls had detectable serum antibodies to squalene. The majority of symptomatic GWS patients had serum antibodies to squalene.

INTRODUCTION

The illnesses afflicting men and women who served in the military conflict in the Persian Gulf during 1990–1991 remain ill-defined. A constellation of symptoms including fatigue, rashes, headaches, arthralgias, myalgias, lymphadenopathy, diarrhea, memory loss, autoimmune thyroid disease, increased allergies and sensitivities to environmental elements, and neurological abnormalities collectively referred to as Gulf War Syndrome (GWS) have been described in veterans from this conflict (Persian Gulf Veterans Coordinating Board, 1985; Grady *et al.*, 1998; Fukuda *et al.*, 1998; Unwin *et al.*, 1999; Coker *et al.*, 1999). A symptom-based case definition of GWS has recently been described (Fukuda *et al.*, 1998). While GWS patients in general do not suffer from classic rheumatic diseases, the signs and symptoms are reminiscent of entities, such as arthralgias, fibromyalgia, lymphadenopathy, autoimmune thyroid disease, chronic fatigue syndrome, malar rashes, and musculoskeletal signs and symptoms associated with various autoimmune conditions and exposure to silicone, an organic material developed, in part, to be used as an immunological adjuvant for vaccines (Ismail *et al.*, 1999; Straus, 1999; Hyams *et al.*, 1996). Many, if not most, of these signs and symptoms are caused, promoted, or modulated by cytokines (Dinarello, 1988; Akiro *et al.*, 1990), further details of which are beyond the scope of this paper. Serological abnormalities including hypergammaglobulinemia and abnormal serum proteins have been reported in 45% of GWS patients (Grady *et al.*, 1998). A variety of possible explanations for GWS have been proposed. The Persian Gulf Veterans Coordinating Board addressed the issues of possible chemical and biological

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0014-4800/00 $35.00

weapons to account for these illnesses (Persian Gulf Veterans Coordinating Board, 1995). Haley *et al.* (1997) grouped various reported symptoms into six different syndromes based upon self-reported possible exposure to chemicals in the Persian Gulf. It has been suggested that a combination of chemical and biological weapons exposure may account for GWS illnesses. Abou-Donia *et al.* (1996) examined the acute effects of pyridostigmine bromide and organophosphate exposure in chickens and suggested that the toxicity observed may be similar to that suffered by Gulf War veterans. Another explanation for GWS is that it is posttraumatic stress syndrome (Hyams *et al.*, 1996).

It has also been suggested that GWS may be due to exposure to biological weapons, dysregulation of the immune system (Rook *et al.*, 1998), or imbalance in the TH1/TH2 ratio, either as an adverse reaction to the intense vaccination schedule or as a result of exposure to biological agents in the Persian Gulf (Rook *et al.*, 1998).

Gulf War veterans and attendant civilian personnel received a variety of immunizations in preparation for possible deployment to the Persian Gulf theater. A similar intensive vaccination regimen was also used in British troops (David *et al.*, 1997). Epidemiological studies indicate that multiple vaccinations or vaccination against biological warfare agents are the factors with the highest correlation with GWS symptomatology (Unwin *et al.*, 1999).

We have identified a group of GWS patients who served in American and British military forces or worked as civilian employees to the U.S. military or their contractors during Desert Shield/Desert Storm in the Persian Gulf, 1990–1991. These patients served in all branches of the military and received the required immunizations. They served throughout the Persian Gulf, including on ships of the U.S. Navy not in combat or exposed to environmental toxins at ground level. We have found antibodies to squalene, an experimental immunological adjuvant, in a high percentage of these GWS cases.

MATERIALS AND METHODS

Patients were admitted to the study based upon service in the United States or the United Kingdom military or as civilian employees of the U.S. military or their contractors in the Persian Gulf during 1990–1991. Patients became aware of the study via the Internet and word of mouth with other veterans and were enrolled consecutively on a voluntary basis. No fees were paid by the subjects or to the subjects who participated in this study. Included were individuals who fit the recently proposed case definition for GWS (Fukuda *et al.*, 1998) and others without GWS symptoms. Service occurred in Desert Shield/Desert Storm, Operation Provide Comfort (in northern Iraq where there were no chemical weapons), CENTCOM in Saudi Arabia, Kuwait, Camp 4 (front lines), and medical units in various locations in Saudi Arabia. Some were in theater for months. Others were evacuated due to illness after as little as 48 h after arrival and before the war commenced. We tested deployed personnel who served in various parts in theater during the war, but were and are not sick. We tested patients referred to as nondeployed veterans, those immunized for duty in the Persian Gulf, but who did not leave the United States or were deployed elsewhere. None participated in NIH experimental vaccination trials, although our positive control subjects had participated in such trials and were known to have received squalene-containing adjuvant injections. Further controls had idiopathic autoimmune disease or silicone breast implants or were healthy subjects with no stigmata of autoimmune disease.

Patient records and histories were obtained from the Gulf War-era participants. Board-certified rheumatologists, neurologists, and endocrinologists made all diagnoses. Compilation of data, including commercial lab results, was done by chart review by one investigator (P.B.A.) and was reviewed by board-certified rheumatologists.[2] Serum samples from study participants were collected by laboratory personnel via standard phlebotomy procedures using vacutainer tubes and butterfly needles and were stored at −20°C until they were shipped to Tulane University School of Medicine in New Orleans. Samples from Gulf War-era veterans were blinded. The identities or exact number of samples from each category was not made available to the Tulane laboratory until after completion of the diagnostic testing. All samples were tested twice under the same conditions. Results from all samples in both tests were consistent. At the end of the study, patient data were matched with the outcome of the anti-squalene antibody (ASA) assay and results were tabulated.

Anti-squalene Antibody Assay

The ASA assay measures the binding of serum immunoglobulin (IgG) to squalene immobilized on nitrocellulose. It is similar in format to the antipolymer antibody (APA) assay

[2]Dr. D. Kevin Asa, M.D., Memphis, Tennessee. Dr. Michele Petri, Johns Hopkins University, Baltimore, Maryland.

for partially polymerized acrylamide (Tenenbaum *et al.*, 1997). Seropositivity on the APA assay has been shown to correlate with severe musculoskeletal signs and symptoms present in a subset of silicone breast implant recipients (Tenenbaum *et al.*, 1997). For the blinded study, squalene (>99% purity) was diluted 10-, 100-, 1000-, and 10,000-fold in distilled water, applied to nitrocellulose membranes, and allowed to air-dry. The nitrocellulose membranes were then cut into 4-mm-wide strips, placed in 20-well trays, and rinsed in wash buffer (Tris-buffered saline containing 0.3% polyoxyethylene sorbitan monolaurate and 0.005% thimerosal, pH 7.4). The strips were incubated in 2 ml blocking buffer (Tris-buffered saline containing 5% powdered instant milk, 4% goat serum, and 0.008% thimerosal, pH 7.4) for 45 min prior to the addition of 5 μl of patient sera (1:400 dilution) followed by a further 90-min incubation. This dilution factor was chosen based upon the very strong antibody responses found in GWS patients. All incubations and washes were carried out at room temperature on a rocking platform. The blocking buffer was then removed and the strips were washed with washing buffer (three times for 5 min each). After the strips were washed, 2 ml of blocking buffer containing biotin conjugated to goat anti-human IgG (Kirkengaard & Perry Laboratories, Gaithersburg, MD), diluted 1:1000, was added. After a 60-min incubation, the strips were again washed as above, and 2 μl of blocking buffer containing avidin-conjugated horseradish peroxidase (Jackson ImmunoResearch, West Grove, PA), diluted 1:500, was added. Following another 60-min incubation, the strips were washed, as above, and 2 ml of detection-buffered saline containing 30% methanol, 0.6 mg/ml 4-chloro-1-napthol, 0.03% hydrogen peroxide; pH 7.4) was added. The reaction was allowed to proceed for 15 min and was stopped by rinsing the strips in distilled water. The strips were allowed to air-dry for visual scoring on a scale of 0 to +4.

Statistical Analysis

The strength of binary relationships was tested using χ^2 tests of independence. This protocol was a feasibility study. Accordingly, no power studies were performed.

RESULTS

Primary Studies

To ascertain that our assay could detect antibodies to squalene, we had positive controls who were two subjects who had volunteered to participate in a vaccine trial at the NIH involving the use of a squalene-containing adjuvant (Table 1). Subsequent to vaccination, they developed a multisystem disorder similar to that of Persian Gulf veterans. Their symptoms are listed in Table 2.

TABLE 1

Squalene Reactivity of NIH Vaccine Trial Participants

Patient	Doses of squalene	ASA reactivity
A	1	+
B	3	+

Patient A received a single injection and became ill within 3 weeks, with signs and symptoms including arthritis, fibromyalgia, lymphadenopathy, photosensitive rashes, fatigue, headaches, and fasciculations. This patient had lower than normal acetylcholinesterase and histological evidence of IgG-mediated demyelinization. The NIH vaccine study code was broken; only adjuvant containing squalene had been administered as a placebo. This patient was weakly positive for ASA. Patient B went through the complete experimental vaccination protocol before manifesting a similar set of signs and symptoms and was +3 for ASA.

Fukuda and co-workers (1998) have reported that individuals deployed to the Persian Gulf who became sick have a chronic multisystem disease. The cohort of GWS patients in our study have many signs and symptoms of autoimmune connective tissue and neurological disease with arthritis (94%), fibromyalgia (94%), lymphadenopathy (94%), rashes (94%), weakness (86%), fatigue (81%), chronic headaches (78%), and memory loss (72%) as the most frequent symptoms (Table 3).

It should be noted, however, that most patients did not have

TABLE 2

Symptoms Which Appeared after a Single Adjuvant Injection

Arthritis
Fibromyalgia
Lymphadenopathy
Rashes
Photosensitive rashes
Malar rashes
Chronic fatigue
Chronic headaches
Fasciculations
Lymphocytic infiltrates around vascular tissue
IgG-mediated demyelinization
Lower than normal levels of acetylcholinesterase

TABLE 3
Symptoms and Diagnostic Lab in GWS Patient Groups

	D-S (%)	D-W (%)	ND-S (%)	UK-D (%)
Arthritis	94	0	100	100
Fibromyalgia	94	8	100	100
Lymphadenopathy	94	0	100	100
Rashes	94	0	100	100
Photosensitive rashes	25	0	75	100
Malar rashes	17	0	63	100
Chronic fatigue	81	33	100	100
Chronic headaches	78	0	100	100
Abnormal body hair loss	19	0	38	33
Nonhealing skin lesions	42	0	63	66
Apthous ulcers	36	0	63	66
Dizziness	47	8	100	66
Weakness	86	17	100	66
Memory loss	72	25	100	66
Seizures	14	0	50	66
Mood changes	72	0	63	100
Neuropsychiatric problems	44	0	88	66
+FANA	20	0	50	Unknown
Anti-dsDNA	14	0	Unknown	Unknown
Low C3 and C4	14	0	Unknown	Unknown
Anti-thyroid	14	0	Unknown	Unknown
Anemia	14	0	50	Unknown
Elevated ESR &/or CRP	25	0	75	Unknown
SLE	17	0	50	Unknown
MS	3	0	Unknown	Unknown
ALS	8	0	0	0
Raynaud's phenomenon	42	0	75	66
Sjogren's syndrome-	8	0	Unknown	33
Chronic diarrhea	36	0	63	66
Night sweats	36	0	88	66
Low grade fevers	39	0	88	66

Note. D-S, deployed, sick (N = 35); D-W, deployed, well (N = 12); ND-S, nondeployed, sick (N = 8); UK-D, deployed, sick, UK (N = 3).

an optimal workup for connective tissue and neurological autoimmune diseases because of the limited resources in the Veterans' Administration hospitals or military hospitals. Nevertheless, all patients reported here meet the case definition recently established (Fukuda *et al.*, 1998). In agreement with a prior study (Grady *et al.*, 1998), some of these GWS patients also had abnormal laboratory values, including positive antinuclear antibodies (ANA;17%), anti-dsDNA (14%), low C3 and C4 (14%), anemia (14%), anti-thyroid microsomal antibodies (14%), and elevated ESR and/or CRP (22%). A minority of symptomatic patients met diagnostic criteria for classical autoimmune diseases, including Sjogren's syndrome (8%), multiple sclerosis (3%), ALS (8%), and systemic lupus erythematosus (17%).

Likewise, military personnel from the United Kingdom have shown the same array of signs and symptoms as those from the United States. Their signs and symptoms included arthritis (100%), fibromyalgia (100%), lymphadenopathy (100%), rashes (100%), chronic fatigue (100%), chronic headaches (100%), and memory loss (66%). Laboratory data are not unavailable for this group. They also had malar rashes, Raynaud phenomenon, and sicca syndromes. Thus, our cohort represents a subset of veterans that displays manifestations of GWS. The severity of symptoms in our cohort can be explained by a self-selection bias in that the patients volunteered for our study.

Persons activated to deploy who were vaccinated, but did not deploy for a variety of reasons, had an array of signs and symptoms with even higher frequencies of arthritis (100%), fibromyalgia (100%), lymphadenopathy (100%), rashes (100%), weakness (100%), fatigue (100%), chronic headaches (100%), and memory loss (100%) (Table 3). The nondeployed individuals had higher rates of dizziness (100%), seizures (50%), and neuropsychiatric abnormalities (88%). The number in this group was small, and these differences were not statistically significant. Laboratory values for the nondeployed individuals with GWS were abnormal, with positive ANA (50%), anemia (50%), and elevated ESR and/or CRP (75%).

In contrast, abnormal signs, symptoms, and laboratory values were rare in the cohort of Gulf War-era veterans who considered themselves well and upon examination did not have debilitating health problems. They reported some signs and symptoms, but their illnesses were not multisystemic (Table 3). The signs and symptoms reported included fibromyalgia (8%), chronic fatigue (33%), weakness (17%), memory loss (25%), and thyroid disease (8%). None reported positive laboratory values for autoimmune processes or were so diagnosed.

Musculoskeletal signs and symptoms are more common in females than males, and autoimmune diseases are predominantly found in females in ratios ranging from 8:1 to 14:1 (Michet *et al.*, 1985; Geirsson *et al.*, 1994). We wished to determine why predominantly male military personnel, both deployed and nondeployed, initially found fit for duty during the war, would develop signs and symptoms common to autoimmune diseases. Many studies have shown that adjuvants used to enhance vaccine efficacy can induce autoimmune disease (Zamma, 1983; Lorentzen *et al.*, 1995; Madzhidov *et al.*, 1986; Kleinau *et al.*, 1995). Thus, we sought whether GWS patients who received immunizations had antibodies to an immunological adjuvant. Squalene was chosen as it has been used in many experimental vaccine adjuvant formulations since 1987. A variation of a previously

described assay, one which measures the binding of serum antibodies to low-molecular-weight polymers (Tenenbaum *et al.*, 1997), was used in the current study. This immunological assay, similar in format to Western immunoblotting, quantitates the binding of antibodies to squalene immobilized on nitrocellulose (Fig. 1). Serum samples were tested blindly. We found that GWS patients who deployed had ASA responses ranging in intensity from +1 to +4. Most of the sick Gulf War veterans had +2 and +3 reactivity to squalene at a serum dilution of 1:400. One individual had an especially strong reaction rated as +4. A high majority (95%) of symptomatic deployed individuals with GWS were positive on the ASA assay (Fig. 2A).

Interestingly, all sick veterans who did not deploy but had received immunizations as preparation for deployment also had antibody reactivity to squalene. In contrast, none of the persons deployed to the Gulf who thought of themselves as well were ASA positive.

Other Studies

Squalene is an organic polymer, with some antigenic epitopes which might be shared with other organic polymers, acting as immunostimulants. Antibodies to silicone and partially polymerized acrylamide (the antigen in the antipolymer assay) were weakly positive in fewer than 10% of the symptomatic Gulf War-era veterans. Four patients with musculoskeletal signs and symptoms and exposure to silicone breast devices were tested to see if antibodies to squalene were present; none were reactive (see below). To determine if antibodies to squalene occurred in idiopathic autoimmune diseases, samples were taken from patients who had defined autoimmune diseases, both rheumatic and neurologic, but none were reactive. To determine if healthy individuals from the general public might have antibodies to squalene, we tested members of the general public. Again, none showed antibody reactivity (Table 4).

In a broader unblinded antibody-screening study, antibodies to squalene were studied in larger groups of individuals (Fig. 2B). Blood samples of Gulf War veterans from different medical centers were tested for ASA. This group contained a high percentage of ASA-positive individuals (69%). The samples included were not segregated according to their clinical status and included healthy controls. Squalene is in some cosmetic products, so we tested to determine if antibodies were present in the general population. Samples of blood from blood banks indicated only 5% antibody reactivity to squalene and the reactions were much less intense (Fig. 1). To determine if antibody to squalene was a marker for autoimmune disease processes, tests were conducted on blood samples from patients with systemic lupus erythematosus. This group had 10% ASA weakly positive reactivity (Fig. 2B). Patients suffering from chronic fatigue syndrome have some of the signs and symptoms of GWS patients, but showed only 15% weak reactivity. Prior studies have shown that most individuals exposed to silicone breast devices with

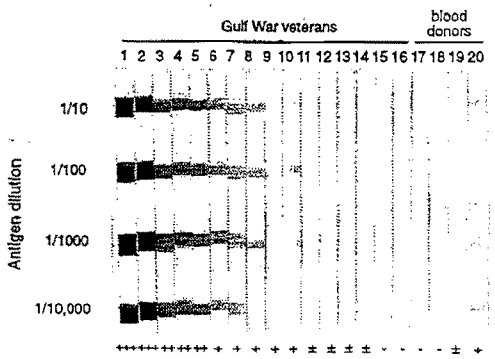

FIG. 1. Antisqualene antibody responses in representative Gulf War Syndrome patients and blood donors.

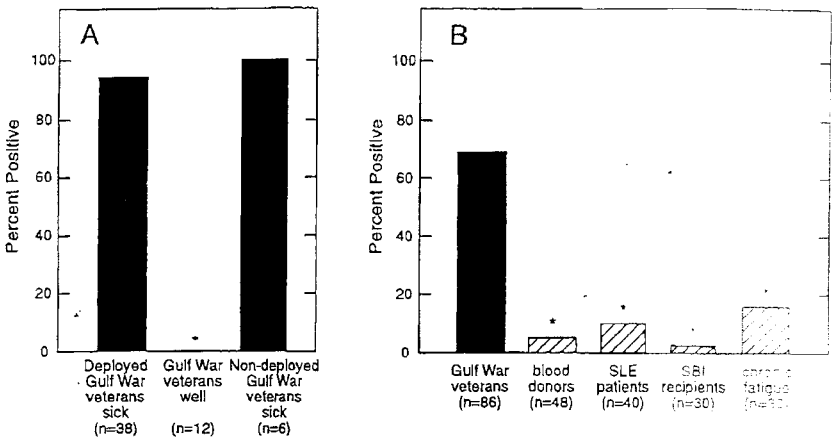

FIG. 2. Antisqualene antibody responses in Gulf War Syndrome patients, blood donors, systemic lupus erythematosus (SLE) patients, chronic fatigue syndrome patients, and symptomatic silicone breast implant (SBI) recipients. (A) Blinded samples. *, $P < 0.001$ compared to percentage positive in well Gulf War veterans by χ^2 test. (B) Unblinded samples. *, $P < 0.001$ compared to percentage positive in Gulf War Syndrome patients.

severe musculoskeletal signs and symptoms have serum antibodies reactive to a synthetic polymer (polyacrylamide) (Tenenbaum *et al.*, 1997). Both silicone and acrylamide, like squalene, are potent immunological adjuvants (Naim *et al.*, 1997; Nicholson *et al.*, 1996; Yoshida *et al.*, 1994; Sergon *et al.*, 1986). Therefore, we tested for cross-reactive antibodies to squalene in serum from patients exposed to SBI. Only 10% of this group were weakly positive for antibodies to squalene (Fig. 2B), confirming the results with the smaller sample in the blinded portion of the study.

TABLE 4
Squalene Antibodies—Blinded Study Patient Groups

Patient group	ASA reactivity (%)
D-S	95
D-W	0
ND-S	100
UK-D	100
Breastimplants	0
NIH vaccine participants	100
Idiopathic autoimmune disease	0
Healthy general public	0

Note. D-S, deployed, sick ($N = 38$); D-W, deployed, well ($N = 12$); ND-S, nondeployed, sick ($N = 8$); UK-D, UK deployed, sick ($N = 3$); NIH vaccine trial patients ($N = 2$).

DISCUSSION

The illnesses afflicting military veterans and civilians who served in the Persian Gulf in 1990–1991 have remained clouded in confusion and controversy. Several recent studies have indicated that the Gulf War-era patients are suffering from a chronic multisystemic illness, but with a continuum of signs and symptoms not within the definitions of "classic" rheumatic diseases or other specific disorders (Fukuda *et al.*, 1998; Ismail *et al.*, 1999; Straus, 1999). In some, onset of illness occurred within a few weeks after receiving immunizations. This includes personnel never deployed due to illness. It also included some who did deploy, but were in theater for as little as 48 h before being sent home before the war began because of severe joint and muscle pain and neurological problems. Other Gulf War veterans became ill

years after the war, but showed illnesses similar to those who became ill soon after vaccination. The variability of expression of symptoms and severity may be due to individual immune responses genetically regulated by the histocompatibility complex (Lorentzen *et al.*, 1995; Madzhidov *et al.*, 1986).

Our results suggest that ASA reactivity is a marker for the signs and symptoms of GWS. Finding serum antibodies to squalene in Gulf War patients is unexpected, and the basis for the presence of these antibodies remains unclear. ASA are not a general marker for autoimmune disease due to their absence in idiopathic autoimmune patients and rarity in patients with other, presumed environmentally induced, autoimmune diseases. The signs and symptoms of our Gulf War patients are similar to those of a subset of female patients following exposure to silicone. Some individuals with silicone exposure suffer from many of the multisystem symptoms, viz, arthralgias, myalgias, lymphadenopathy, and neurological disorders prevalent in GWS patients in the current study (Bridges *et al.*, 1993; Braunbar *et al.*, 1995; Wolford, 1997). Symptomatic silicone breast implant recipients also have high levels of antibodies to synthetic polymers (Tenenbaum *et al.*, 1997) and to silicone,[3] but did not have high prevalence of ASA.

It has been suggested that abnormal immune responses may be involved in GWS (Rook *et al.*, 1997). Immunological adjuvants have the generally desirable property of eliciting cell-mediated immunity and antibodies when administered with an antigen. They may also cause a more generalized and indiscriminate stimulation of the immune system and disrupt the balance of immune self-regulatory mechanisms, which may lead to autoimmune disease (Zamma, 1983; Lorentzen *et al.*, 1995; Madzhidov *et al.*, 1986; Kleinau *et al.*, 1995). Squalene has been used extensively as an adjuvant in animal models to induce autoimmune diseases (Lorentzen, 1999; Beck *et al.*, 1976; Kohashi *et al.*, 1977; Garrett *et al.*, 1985; Whitehouse *et al.*, 1974; Yoshino *et al.*, 1994). Cytokines are mediators of immunological regulation and inflammatory responses (Van der Meide *et al.*, 1996), and increased cytokine levels are associated with the development of autoimmune disease in established rodent models of autoimmunity (Fitzpatrick *et al.*, 1996). Squalene has been shown to induce increased levels of interleukin-5 (IL-5), IL-6, and interferon-γ (Valensi *et al.*, 1994). Several different adjuvants have been demonstrated to produce or exacerbate autoimmune diseases in experimental models. Adjuvant-induced arthritis is a well-characterized autoimmune disease induced in rats and other species (Zamma, 1983; Lorentzen *et al.*, 1995; Madzhidov *et al.*, 1986; Kleinau *et al.*, 1995). The disease process in adjuvant-induced arthritis is complex, affecting multiple organ systems. For example, a cachetic syndrome (Rofe *et al.*, 1994) and testicular dysfunction (Clemons *et al.*, 1988) have been associated with adjuvant-induced arthritis. Uveitis, a T-cell mediated intraocular inflammatory disease, can also be induced by adjuvants (Petty *et al.*, 1996). Neurological diseases can be the result of immunological mechanisms including autoimmunity (Rogers *et al.*, 1996; Tebin *et al.*, 1996; Honnorat *et al.*, 1995; Wucherpfennig *et al.*, 1990; Cross *et al.*, 1991; Bansal *et al.*, 1994), and neurological symptoms are commonly seen in autoimmune diseases (McNicholl *et al.*, 1994; Zanone *et al.*, 1993; Moll *et al.*, 1993).

All pharmacology is controlled toxicology. Although not approved by the Food and Drug Administration for human use, squalene has been used as an adjuvant in experimental vaccines against a variety of pathogens, including *Bacillus anthracis* (Ivins *et al.*, 1994), *Plasmodium falciparum* (Hoffman *et al.*, 1994), and herpes simplex virus (Burke *et al.*, 1994). Effectiveness of adjuvants has been shown to parallel toxicity defined as the initiation of autoimmune disease processes (Zamma, 1983; Koga *et al.*, 1986). Adjuvants should not produce reactions at injection sites, be pyrogenic, or induce anterior uveitis, arthritis, or other protean autoimmune processes (Allison *et al.*, 1991). A study using squalene as an adjuvant in influenza vaccine reported moderate to severe local and systemic reactions in humans (Keitel *et al.*, 1993). The participants suffered induration, erythema, lymphadenopathy, fever, chills, nausea, and dizziness, symptoms which lasted for several days. Another squalene-containing adjuvant was used with gp120 in a human immunodeficiency virus vaccine, where it induced severe systemic and local reactions in 15 of 30 vaccinees (Keefer *et al.*, 1996). Similarly, in a study of simian immunodeficiency vaccine in macaques, squalene was used as an adjuvant, and the animals developed anti-human-cell antibodies and autoimmune-like symptoms (Vaslin *et al.*, 1992). Future studies should determine whether or not ASA have a role in these pathological processes.

Squalene is a naturally occurring molecule absorbed from food and synthesized as a precursor for cholesterol, myelin, and hormones. This synthesis occurs within the hepatocytes and is further processed into cholesterol in the endoplasmic reticulum (Stamellos *et al.*, 1993). Fecal analysis indicates that about 60% of dietary squalene is absorbed (Strandberg *et al.*, 1990). Dietary squalene is absorbed through lymphatic vessels after being cyclized to sterols during transit through

[3]Cao, Yan *et al.*, unpublished observations.

the intestinal wall (Tilvis *et al.*, 1983). It is processed into chylomicrons by the epithelial cells of the small intestines. It becomes a lipid droplet covered by β-lipoprotein containing triglyceride and cholesterol ester. This increases serum levels of free and esterified methyl sterol contents. About 90% of absorbed squalene is in lipoproteins, appearing in chylomicrons and VLDL, suggesting that removal of dietary squalene may indicate metabolism of intestinal lipoproteins (Gylling *et al.*, 1994).

Squalene is a nonsteroid precursor of cholesterol. Reports have indicated that high titers of autoantibodies to cholesterol, once considered to be a poorly immunogenic molecule, could be generated by immunization with liposomes containing cholesterol and lipid A as adjuvant (Swartz *et al.*, 1988; Alving *et al.*, 1991; Dijkstra *et al.*, 1996). Injection of either silicone gel or silicone oil intraperitoneally also resulted in high titers of autoantibodies to cholesterol (Alving *et al.*, 1996). The silicone component serves as an adjuvant as well as initiating the autoimmune process. The high titers were IgM with relatively low titers of IgG to cholesterol (Dijkstra *et al.*, 1996; Alving *et al.*, 1996). The specificity of these antibodies was to cholesterol and structurally similar sterols containing a 3β-hydroxyl group. Anticholesterol binding activity was significantly diminished if the 3β-hydroxyl domain was altered by oxidation, substitution, epimerization, or esterification (Dijkstra *et al.*, 1996). It has been reported that naturally occurring autoantibodies have been detected in humans (Alving *et al.*, 1989), but these were much lower in titer than those produced with either lipid A or silicone.

Several facts argue against our assay detecting cross-reactive antibodies to cholesterol instead of antibodies specific for squalene. First, squalene is neither a sterol nor does it have a 3β-hydroxyl group. The respective molecular structures, internal molecular bonding, charge distribution, and antigenic epitopes are different. Second, if high-titer autoantibodies to cholesterol that are cross-reactive with squalene are normal, we should see no difference between our various patient groups. The GWS patients and NIH positive control patients are very distinct in their strong IgG antibody reactivity to squalene. Third, if silicone alone can generate antibodies to cholesterol and these are cross-reactive to squalene, we should see high antibody reactivity to squalene in patients exposed to silicone in addition to the GWS and NIH patients. This did not occur.

In the course of these studies, we examined two volunteers for a vaccine trial at the NIH involving squalene as adjuvant. They developed a multisystem disease similar to that seen in Persian Gulf veterans subsequent to their participation in the trial. One received a single injection and became ill within a few weeks with signs and symptoms including arthritis, fibromyalgia, lymphadenopathy, photosensitive rashes, fatigue, headaches, and fasciculations. This individual had lower than normal acetylcholinesterase, histological evidence of lymphocytic infiltrates around vascular tissue, and IgG-mediated demyelinization. After this NIH vaccine study code was broken, it was found that only adjuvant squalene had been administered as placebo. This patient was weakly positive for ASA. Another patient who went through the whole experimental protocol before manifesting a similar set of signs and symptoms was 3+ positive for ASA.

Multiple vaccinations and vaccination against biological warfare agents are the factors with the highest correlation with GWS symptomatology (Unwin *et al.*, 1994). It is important to note that our laboratory-based investigations do not establish that squalene was added as adjuvant to any vaccine used in military or other personnel who served in the Persian Gulf War era. Several investigators have speculated that GWS is the result of either exposure to chemicals, chemical weapons, or to biological agents encountered in the Persian Gulf (Persian Gulf Veterans Coordinating Board, 1995; Abou-Donia *et al.*, 1996; David *et al.*, 1997; Haley, 1997). However, such exposure would likely have immediate effects and many Gulf War veterans were well until months or years after the military conflict. Many of these GWS patients have improved on treatment regimens prescribed by their personal physicians, rheumatologists, and neurologists, namely the immunosuppressives used for classical rheumatological conditions.[4] These treatments have included steroids, methotrexate, hydroxychloroquine, and cytoxan. Such treatments would have no effect on subjects exposed to chemical weapons. If GWS was due to an exogenous infectious agent, the immunosuppressive regimens used would likely result in an exacerbation of the symptoms. This did not occur. The molecular pathology of GWS must be defined before its etiology can be assigned. We present here evidence of an immune factor based upon the adjuvancy of squalene. Further studies are required to define the role of ASA, if any, in the pathogenesis of GWS.

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[4] Asa, P.B. *et al.*, unpublished observations.

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Subject: DESERT SHIELD BIOLOGICAL WARFARE HOC WORKING GROUP

Unit: OTSG

Parent Organization: HSC

Box ID: BX003203

Folder Title: MINUTES OF THE DESERT SHIELD BIOLOGICAL WARFARE

Document Number: 1

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protective. A second challenge of these animals, withoi additional antibiotic prophylaxis, found them to be susci to anthrax exposure. The animals who had previously reci e

6. (U) BW Vaccines, Botulinum Antitoxin and IND Drugs Theater.

a. COL Tomlinson reported on an outbreak of foodbor: botulinum in Cairo, Egypt, and that a quantity of the bo@ antitoxin from theater was shipped to Egypt. LTC McKee there were approximately 80 cases; with 15 of these resu in death. The outbreak was believed to be associated wi@ under cooked fish. He also reported individuals from CD, to Egypt to investigate the outbreak and took antitoxin 1 them. LTC McKee stated that in addition to the Army's a: CDC's antitoxin, antitoxin was also supplied by a Europe, manufacturer. Since several sources of antitoxin were u some individuals may have received several doses of diff, antitoxin, evaluation of the efficacy of the Army produc be difficult at best. LM Ld

b. It was reported that the individuals from logist USAMRIID, were expected back from theater today with the anthrax and botulinum vaccines, antitoxin, ribavirin and centoxin. While in theater the items were under refrigeration; however, there was a report that the refrigerator failed to operate for a period of time and possibly these items were damaged. The items will be re to USAMRIID and a determination made with regard to the disposition. I

7. (U) Documentation of Vaccine Usage in Theater.

2D DISTRICT, WASHINGTON

COMMITTEE ON TRANSPORTATION AND INFRASTRUCTURE
SUBCOMMITTEES:
AVIATION
GROUND TRANSPORTATION

COMMITTEE ON SCIENCE
SUBCOMMITTEE:
ENERGY AND ENVIRONMENT

Congress of the United States
House of Representatives
Washington, DC 20515–4702

COMMITTEE ON BANKING AND FINANCIAL SERVICES
SUBCOMMITTEES:
HOUSING
FINANCIAL INSTITUTIONS
DOMESTIC AND INTERNATIONAL MONETARY POLICY

CHAIR, REPUBLICAN HOUSING OPPORTUNITY CAUCUS

REPUBLICAN POLICY COMMITTEE

May 13, 1999

The Honorable William S. Cohen
Secretary of Defense
The Pentagon
Washington, DC 20301-1010

Reference: "Questions About the Presence of Squalene Antibodies in Veterans Can Be Resolved. GAO, March 1999

Dear Secretary Cohen:

On March 29, 1999, the General Accounting Office released the report I had requested regarding squalene antibodies in veterans suffering from Gulf War Illnesses. As DOD prepares its response to the final report issued by the GAO, I am requesting answers to a number of questions that remain outstanding.

In the report DOD commented, "There is no basis for believing that Gulf War-era veterans were exposed to squalene-containing vaccines. The DOD has indicated that no experimental vaccines with squalene containing adjuvants had been used in U.S. troops during the Gulf War." (Page 23)

Contrary to the above assertion, the GAO report did not implicate the Department of Defense. Rather, the report concluded it would be prudent for DOD to "review the independent research that researchers report has revealed the presence of squalene antibodies in the blood of ill gulf War-era veterans, and conduct its (DOD) own research designed to replicate or dispute these results." (Pg 8-9)

1. DOD officials told the GAO, that DOD could develop an assay for detecting antibodies to squalene, and a sample testing could be done for a small investment. Will the DOD reassess their former position, and aggressively pursue this first step? Determining if the antibodies are present is vitally important. If they are present, then the process to ascertain the significance of that finding can begin.

2. If the DOD is concerned that it does not have the resources, or that it would require a lengthy period of time (over six months) to conduct an initial investigation, is there a reason why you cannot send a team of experts to Tulane University where the research has been done to validate or dispute its integrity?

3. In light of the missing shot records of so many of our Gulf War-era veterans, is it possible to determine absolutely that they did not receive any vaccine formulations containing squalene during or prior to the Gulf War? Is this conclusion based solely on the statement of the vaccine manufacturer?

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The DOD stated in its response, "The assay for anti-squalene antibodies developed by the independent researchers has not been validated through peer review or publication in the scientific literature . . . Data obtained from a methodology that has not been validated have significant potential to harm or mislead Gulf War veterans through the medical misinformation the data may support." (Page 23)

The researchers at Tulane have made clear their willingness to work with DOD. Time is critical for thousands of sick Gulf War-era veterans who continue to suffer and have been waiting the last seven years for help. The truth cannot harm or mislead Gulf War veterans. You have the capability to validate or dispute the methodology.

However, not getting to the bottom of this perplexing problem will no doubt continue to have serious ramifications. I am sure you are aware of the growing concern among active military members regarding the current anthrax vaccination program. Reports of serious adverse reactions are increasing. The oversight hearing of the Subcommittee on National Security, Veterans Affairs, and International Relations on April 29, 1999, revealed troubling testimony. Members of the Michigan Air National Guard are suffering significant health consequences following their anthrax vaccinations. During the hearing, the GAO raised a number of critical questions regarding the safety and efficacy of the anthrax vaccine. Combined with the squalene issue, these factors are escalating a climate of distrust. Inaction, while waiting for the lengthy peer review process, will only exacerbate this disturbing situation.

4. Confirmation exists that several active duty personnel recently inoculated have tested positive for antibodies to squalene. Several publications have alleged a potential connection between anthrax vaccinations and squalene. Therefore, is it not in the best interest of the United States active duty forces to immediately take action to determine the facts and potential health consequences?

This situation provides the DOD an extraordinary opportunity to demonstrate our nation's committment to the honorable men and women who serve our country. Thank you for your assistance. I look forward to your personal reply.

Sincerely,

Jack Metcalf

Jack Metcalf

Tulane University Medical Center

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Robert F. Garry, Ph.D.
Professor

8/14/00

RE: Note to file regarding conversation with COL Carl R. Alving, M.D.

To whom it may concern:

On or about May 24, 1999 I received an unsolicited telephone call at my office from COL Carl R. Alving, M.D. Dr. Alving, whose work on lipids and adjuvants I was somewhat familiar with, indicated that he had a scientific interest in the work on squalene antibodies conducted by Drs. Pamela Asa and Yan Cao and myself.

Dr. Alving indicated that his interest in my studies was "purely scientific" and that he wished to get more information because of his interest in the general area of lipids, antibodies and adjuvants. This was plausible because of Dr. Alving's prior work in this general area. The conversion, which lasted from about 45 minutes, was almost entirely scientific and covered a broad range of topics related to anti-lipid antibodies.

During the course of our conversation, Dr. Alving shared some of his recent studies on anti-cholesterol antibody with me. At that time, I was only vaguely familiar with those studies. Dr. Alving offered the opinion that the anti-squalene antibodies might be a subclass of the anti-cholesterol antibodies. I replied that this might be worth looking into.

Dr. Alving also asked to review a draft of the manuscript on anti-squalene antibodies which was subsequently published in *Experimental and Molecular Pathology*. I agreed to fax him a copy of the *in progress* work for his personal review. Because the work had not yet been accepted for publication, I asked that he not circulate the copy.

At no time was I made aware that Dr. Alving's intent was to circulate our paper and subject it to the scathing reviews subsequently published on the DoD website prior to publication and in abbreviated form as a letter to the editor of *Experimental and Molecular Pathology* .

Robert F. Garry

Robert F. Garry, Ph.D.
Professor

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May 25, 1999

Col. Carl Alving, M.D.
Department of Membrane Biochemistry
Walter Reed Army Institute of Research
Building 40, Room 1022
Washington, DC 20307-5100

Dear Dr. Alving:

It was a pleasure talking with you today. As we discussed, I am enclosing two reprints and a copy of a manuscript that deal with our work on anti-polymer antibodies. I would appreciate any comments or questions that you might have concerning our work.

In regards to the anti-squalene antibody assay, I mentioned to you that Tulane University Medical Center has filed for patent protection concerning the use of anti-squalene antibodies in evaluating Gulf War Syndrome. As Tulane's exclusive licensee for this technology, we would be happy to discuss information regarding the assay and our research with you. If you need additional information or have any questions, please contact me.

By the way, I wanted to mention to you that I have read many of your papers concerning liposomes and toxins. My dissertation project many years ago, concerned the cloning of exotoxin A from *Ps. aeruginosa*, and after our phone conversation, I remembered reading your paper on the interaction of diphtheria toxin and phospholipids. Again, it was a pleasure talking with you today, and I look forward to talking with you soon.

Sincerely,

Russell B. Wilson

Russell B. Wilson, Ph.D.
President

enclosures

THE ASSISTANT SECRETARY OF DEFENSE
WASHINGTON, DC 20301-1200

HEALTH AFFAIRS

28 MAY 1999

Mr. Kwai-Cheung Chan
Director, Special Studies and Evaluations
National Security and International Affairs Division
U.S. General Accounting Office
Washington, DC 20548

Dear Mr. Chan:

This is the Department of Defense (DoD) response to the General Accounting Office (GAO) final report, GAO/NSIAD-99-5, "GULF WAR ILLNESSES: Questions About the Presence of Squalene Antibodies in Veterans Can Be Resolved," dated March 29, 1999 (GAO Code 713014/OSD Case 1711).

The Department acknowledges receipt of the final report and inclusion of the DoD response to the draft report as Appendix VI. We acknowledge the extensive changes that GAO made to the report based on the published DoD response and the other comments and annotations to the draft report, which we had provided to GAO separately.

Our position and the concerns expressed in our comments to the draft report have not changed. The clinical significance and origin of antibodies to squalene, if their existence is corroborated, remain unknown. The test methods proposed by the investigators at Tulane University need to be reviewed and validated by other scientists. Finally, no vaccines with squalene-containing adjuvants were used in U.S. troops during the Gulf War.

The Department continues to solicit and fund research designed to better understand and treat the health problems of Gulf War veterans. Requests for research proposals are published as Broad Agency Announcements in the Commerce Business Daily and are readily available to interested civilian and Federal investigators. We encourage investigators, including those at Tulane University, to submit research proposals that further our understanding of illnesses among Gulf War veterans. Our commitment to civilian and Federal researchers and to Gulf War veterans is to the support and funding of high quality research, which is best assured when all decisions on research funding are based on a process of rigorous, competitive, and independent peer review of all research proposals.

Sincerely,

Dr. Sue Bailey

ISSUES RELATING TO ANTIBODIES TO SQUALENE

Background

Recently, Pamela B. Asa, Ph.D. (of Memphis, TN) and Robert F. Garry (of Tulane University, New Orleans, LA) have been quoted in the popular press as claiming that a higher percentage of sick Gulf War veterans than healthy Gulf War veterans, or than normal blood donors, have antibodies to squalene in their blood. Squalene is a naturally-occurring, oil (a molecule that is in the category of fats and lipids) that is widely distributed in large quantities in the human body, and that has been proposed for use as a commercial adjuvant for increasing the potency of vaccines. Based on the claims by Drs. Asa and Gary, numerous accusations have been leveled at DoD. These include (among others) the allegation that the U.S. Army spiked the anthrax vaccine with squalene as an adjuvant during the Gulf War, and the claim that antibodies to squalene are responsible for the symptoms observed in sick Gulf War veterans.

A detailed investigation of numerous issues relating to squalene and squalene antibodies has been made by the U. S. Army Medical Research and Material Command (USAMRMC) and the Walter Reed Army Institute of Research (WRAIR). The commanding general of USAMRMC, MG John Parker, personally telephoned Dr. Garry, and also assigned COL Carl R. Alving, M.D. (Colonel, U. S. Medical Corps) to call Dr. Garry and to investigate the technical aspects of squalene and antibodies to squalene. COL Alving, who is Chief of the Department of Membrane Biochemistry at WRAIR, has had more than 30 years of research and clinical experience in studying the biochemistry and immunology of lipids, fats, and oils, and is internationally recognized for his research and clinical experience with lipids and oils as adjuvants for vaccines. He is also one of the world's foremost experts in the study of antibodies to lipids. The comments that follow result from this investigation.

Conversation with Dr. Garry

On Monday 24 May 1999 COL Alving and one of his staff members, Gary R. Matyas, Ph.D. (another expert in biochemistry, lipids, antibodies to lipids, immunology, and oil-based adjuvants), called Dr. Garry to discuss Garry's method of measuring antibodies to squalene. Based on the telephone conversation, at the present time the results claimed by Drs. Asa and Garry that have been made in the popular press have not even been minimally validated by scientific peer review. According to Dr. Garry, an attempt has been made Dr. Asa and him (together with Yan Cao, M.D. of Tulane) to achieve at least some measure of scientific peer acceptance by submitting a paper for publication in the Journal of the American Medical Association. However, to date, this effort has not been successful. Dr. Garry faxed a copy of the manuscript (that was marked as being a "revised" version) to COL Alving. Dr. Garry stated that the manuscript had somehow been published without his permission on the internet and that because of the publicity he doubted that it would be published as a scientific peer-reviewed paper.

Analysis of the purported assay for antibodies to squalene

When Garry was asked to provide a detailed standard operating procedure for detecting antibodies to squalene, he said that the complete details were given in the manuscript that he faxed. However, COL Alving and Dr. Matyas found literally dozens of important technical and theoretical flaws in the assay that was faxed by Dr. Garry. Many of these were fatal flaws. Although many of the flaws that were detected by Alving and Matyas require a detailed technical knowledge of such assays, some can be explained rather simply, as shown below.

First, the conclusions are entirely based on faulty, nonscientific, circular reasoning. Positive results in an unproven assay that has not been previously validated to detect antibodies against an antigen cannot then be used be used as scientific proof that antibodies to the antigen exist in an unknown sample. The assay must first be validated by independent means. In scientific terms, it would be said that there were ***no validated positive controls***.

Second, the assay is notable for its **lack of negative controls**. There is no control in which the human serum containing the presumed antibodies is omitted. There is no control in which the avidin-conjugated horse radish peroxidase is omitted. Finally in a new unproven assay it is essential to prove specificity of the assay. There is no evidence that the assay is not simply measuring nonspecific IgG molecules that are not antibodies to squalene but nonspecifically stick to squalene. Although IgG molecules were detected in the assay with second antibodies to human IgG, there were no controls to show that second antibodies to other normal serum proteins (e.g., albumin, fibrinogen, alpha 2 macroglobulin, complement, etc.) could not also have have been detected. The entire assay may be completely due to nonspecific binding of squalene to IgG molecules that are not actually antibodies.

Third, the unknown human serum samples were **tested only at a single very high dilution** (a dilution of 1/400). Most assays for naturally-occurring antibodies, particularly antibodies to lipids, start at a much higher concentration of serum, typically a dilution of 1/50. Thus, the Garry method would be expected to miss the presence of all of the antibodies that would be detected at a higher concentration of serum. In fact, it is possible that at a higher concentration of serum 100% of normal blood donors might give positive results. [When this was pointed out to Dr. Garry, he admitted that a much higher percentage of positives in normal serum might have been detected with more concentrated serum.] A further drawback of the use of only a single dilution of serum rather than a series of dilutions, is that there is a no way to obtain a titer, i.e., a quantitative measure of the degree of activity in the sample. Titers are routinely obtained in measurement of antibody levels, and the absence of quantitation in the Garry assay prevents any meaningful comparison between unknown serum samples.

Fourth, no specificity controls were run to determine if the antibody binds to other structurally related compounds, such as cholesterol. Although Dr. Garry verbally stated that the antibodies did not bind to squalane (the fully hydrogenated analog that lacks double bonds), there was no evidence of any specificity whatsoever in the manuscript that was sent for peer review. One can only wonder why such important information would

be left out of the first description of an unproven assay that purports to measure specific antibodies.

As stated earlier, numerous other important and fundamental flaws were detected in the assay. This can only lead to the conclusion that even if the paper is ultimately published in its present form, there will continue to be, at the least, considerable controversy over the scientific validity of the assay and the conclusions derived from the assay.

Commercialization of Dr. Garry's assay

On Tuesday, 25 May 1999 COL Alving and Dr. Matyas had a detailed telephone conversation with Dr. Russell Wilson, President of Autoimmune Technologies, L.L.C. (New Orleans, LA). This was done because Dr. Garry had indicated that, even in the absence of peer-reviewed scientific validation, the patent rights to the technology for measuring antibodies to squalene had been exclusively licensed by Tulane University for commercial development by this company. Dr. Wilson confirmed that Drs. Garry and Asa are listed as coinventors on the patent for the assay that has been exclusively licensed by Autoimmune Technologies. This was further confirmed in a letter dated 25 May that was shipped to COL Alving by Dr. Wilson. According to Dr. Wilson, the company does not currently have any type of kit or other product that can be purchased for detecting antibodies, but is in the process of developing a product. Dr. Wilson stated that the company is working on an "ELISA-based version" of the assay. If this is true, then it might represent still another assay that has not been validated in a normal scientific manner.

Financial conflict of interest of Drs. Asa and Garry

The exclusive licensing of the above patent application, on which Asa and Garry are coinventors, to Autoimmune Technologies establishes an obvious, and highly disturbing, economic motive to achieve widespread testing for profit. In the absence of such testing for antibodies to squalene, the exclusive license to Autoimmune Technologies would be worthless. Furthermore, Dr. Wilson stated, and the faxed manuscript confirmed, that Autoimmune Technologies also provides professional financial support for Dr. Garry at Tulane in the form of a grant. Although the issue was not investigated in depth with Dr. Garry or Wilson, it is likely that Drs. Asa and Garry also stand to benefit personally from commercialization of the patent. The financial benefits that would accrue to Drs. Asa and Garry, both professionally and personally, therefore create an obvious conflict of interest that, at a minimum, could be expected to color their scientific objectivity.

Anti-military agenda of Drs. Asa and Garry

It is disturbing to note that the strongest thrust of the above manuscript by Asa, Cao, and Garry, that is based on an unvalidated and unproven assay, is apparently directed to trying to convince sick Gulf War veterans that their illnesses are due to the presence of antibodies to squalene. There is an apparent agenda to convince veterans who put their lives on line in the Gulf War, that such antibodies were actually caused by their Gulf War experience. From the quotations in the popular press it is clear that there is also an agenda by some to claim that the antibodies were

induced by the alleged secret use of squalene as an adjuvant in the anthrax vaccine. To his credit, when asked about this, Dr. Garry stated that he did not believe that the antibodies were caused by any conspiracy to spike the anthrax vaccine with squalene. Instead, he apparently adhers to an alternative, but also unproven, theory that some constituent of the anthrax vaccine exhibits structural homology with squalene, a phenomenon sometimes referred to as molecular mimicry, and that the antibodies were induced by the anthrax vaccine in this manner. None of this is proven. No such structural homolog has ever been identified. This is an untested theory that has no basis whatsoever in fact. The only evidence, if it were viewed as such, is the unvalidated and unproven assay of Garry that purports to detect higher levels of antibodies to squalene in sick Gulf War veterans than in the normal population. The apparent anti-military agenda of Drs. Asa and Garry is a clear factor that could color their scientific results. Because of this, the Army could be made vulnerable by exclusive reliance on collaboration with Dr. Garry or Autoimmune Technologies. There is an obvious need for independent in-house research by the Army to examine the issues and implications, if any, of antibodies to squalene.

Antibodies to lipids are not new or unique: Antibodies to cholesterol in normal human sera

The concept of the presence of antibodies to lipids in human serum is not a new idea. COL Alving is particularly well-known for having discovered that 100% of normal human sera contain naturally-occurring antibodies to cholesterol. This observation was first made in 1988 and has been independently confirmed in the peer-reviewed literature. COL Alving has even created and patented a monoclonal antibody to cholesterol, and the clone is on deposit in the American Type Culture Collection. It has been proposed that naturally-occurring antibodies to cholesterol in humans actually serve a useful and beneficial function in helping to remove low density lipoprotein cholesterol (so-called "bad" cholesterol) from the blood. Because squalene is a precursor and building block for cholesterol in the human body, and is structurally very similar to cholesterol, it is the opinion of COL Alving that so-called antibodies to squalene might actually be antibodies to cholesterol that are cross-reacting with squalene. Thus it is possible that the apparent antibodies to squalene, per se, do not exist but rather are antibodies to cholesterol that have beneficial effects. When this was raised as an issue by COL Alving in his conversation with Dr. Garry, it was obvious that Dr. Garry was completely unaware of the scientific literature that exists on antibodies to cholesterol. When informed of the antibodies to cholesterol, Dr. Garry agreed that the purported antibodies that he observed might very well represent antibodies that react with cholesterol.

Pert Chart for Completion of Studies on Antibodies to Squalene

	FY99		FY00			FY01				FY02				FY01			
	$154,792		$509,256			$596,786				$493,606				$230,398			
Milestones	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51
Development and Testing of ELISA Assay for Antibodies to Squalene																	
Evaluation and Development of Other Assays for Antibodies to Squalene																	
Development of a Positive Control Antibody to Squalene																	
Large Scale Production of a Positive Control Antibody to Squalene for Use in Assays																	
Testing of Normal Human Serum for Antibodies to Squalene by ELISA																	
Testing of Normal Human Serum for Antibodies to Squalene by Other Methods																	
Development of a High Volume Throughput ELISA for the Measurement of Antibodies to Squalene in Serum																	
Development of a High Volume Throughput Assay Using an Alternative Method for the Measurement of Antibodies to Squalene in Serum																	
Testing of Gulf War Veterans for Antibodies to Squalene																	
Determination of Specificity of Antibodies to Squalene																	
Biological Significance of Antibodies to Squalene																	

SUMMARY BUDGET SQUALENE ANTIBODY TASK

Milestones	FY 99				FY 00				FY 01			
	Materials	Personnel	Overhead	Total	Materials	Personnel	Overhead	Total	Materials	Personnel	Overhead	Total
Development and Testing of ELISA Assay for Antibodies to Squalene	10,000	41,280	5,128	56,408	0	41,280	4,128	45,408	N/A	N/A	N/A	N/A
Evaluation and Development of Other Assays for Antibodies to Squalene	10,000	32,960	4,296	47,256	10,000	98,800	10,888	119,768	N/A	N/A	N/A	N/A
Development of a Positive Control Antibody to Squalene	13,520	32,960	4,648	51,128	45,080	98,880	14,396	158,356	N/A	N/A	N/A	N/A
Large Scale Production of Positive Control Antibody to Squalene for Use in Assays	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	15,800	0	1,500	16,500
Testing of Normal Human Serum for Antibodies to Squalene by ELISA and Other Methods	N/A	N/A	N/A	N/A	20,000	37,440	5,744	63,184	10,000	12,480	2,248	24,728
Development of a High Volume Throughput ELISA for the Measurement of Antibodies to Squalene in Serum	N/A	N/A	N/A	N/A	15,000	57,600	7,260	79,860	N/A	N/A	N/A	N/A
Development of a High Volume Assay Using an Alternative Method for the Measurement of Antibodies to Squalene in Serum	N/A	N/A	N/A	N/A	10,000	28,800	3,880	42,680	10,000	28,800	3,880	42,680
Testing of Gulf War Veterans for Antibodies to Squalene	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	50,000	37,440	10,744	118,184
Determination of Specificity of Antibodies to Squalene	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	40,000	131,840	17,184	189,024
Biological Significance of Antibodies to Squalene	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	55,133	131,840	18,697	205,670
Total	33,520	107,200	14,072	154,792	100,080	363,888	46,396	509,256	288,133	342,080	54,253	596,786

Total Cost for First Three Years $1,260,834

THE ASSISTANT SECRETARY OF DEFENSE
WASHINGTON, D. C. 20301-1200

HEALTH AFFAIRS

Honorable Jack Metcalf
United States House of Representatives
Washington, DC 20515-4702

Dear Representative Metcalf:

This is in reply to your letter to Secretary Cohen regarding the United States General Accounting Office (GAO) report, GAO/NSIAD-99-5, "GULF WAR ILLNESSES: Questions About the Presence of Squalene Antibodies in Veterans Can Be Resolved." Thank you for your letter and for your concern for the health and welfare of military members and veterans.

The Department's position and concerns have not changed from those published as Appendix VI of the GAO report. The clinical significance and origin of antibodies to squalene, if their existence is corroborated, remain unknown. The test methods proposed by the investigators at Tulane University need to be reviewed and validated by other scientists. Finally, no vaccines with squalene-containing adjuvants were used in U.S. troops during the Gulf War.

Learning the clinical significance and origin of antibodies to squalene is a more important first step than knowing if such antibodies exist in a given person or group of persons. Well-designed laboratory and animal studies must precede studies in humans to answer these questions.

The forum for validating or disputing the integrity of medical research findings or clinical hypotheses is through subjecting one's work to peer review by scientists through presentation at scientific meetings and publication in peer-reviewed scientific publications. The assay for anti-squalene antibodies, which independent researchers at Tulane University developed, has not been validated at other laboratories nor have their methods and findings been subjected to broad peer review. A draft manuscript reporting the Tulane scientists' methods and findings was provided to the Research Working Group of the Persian Gulf Veterans' Coordinating Board. The Research Working Group is currently evaluating the work, will review other available literature, and will produce a White Paper on the significance of the unpublished findings.

No vaccines with squalene-containing adjuvants were used in U.S. troops during the Gulf War. There was no mention in Gulf War era documents that the DoD ever considered producing or using a vaccine that would not comply with the Food and Drug Administration's requirements for a licensed product or a product in an investigational new drug status. For several years, however, one of the scientists on the Tulane report has speculated that an autoimmune response to a vaccine adjuvant may be the cause of illnesses among Gulf War veterans. The initial speculation was that vaccines given to service members during the Gulf War contained squalene

as an adjuvant. Subsequently, the speculation was that Gulf War era service members received an experimental anti-HIV vaccine containing squalene without their knowledge.

Only recently has the speculation, as presented in the lay press, shifted to theories of adjuvants containing squalene in the anthrax vaccine. The anthrax vaccine did not and does not contain squalene. We are extremely confident of that statement; however, to reassure our service members and the public we have begun testing existing anthrax vaccine lots for the presence of squalene. The independent civilian laboratory conducting the test reports that no squalene was detectable in any vials from the six anthrax vaccine lots that have been tested to date.

The Tulane scientists have been encouraged to submit a research proposal in response to existing DoD broad agency announcements requesting proposals for Gulf War illnesses-related research. If and when the independent researcher or any other scientist submits for funding a research proposal for further studies of the alleged finding of antibodies to squalene, the DoD will ensure that the proposal receives a fair evaluation by the independent scientific review panel, which assesses all such proposals. The Department of Veterans Affairs (VA), Office of Research and Development, also has encouraged the Tulane investigators to identify a VA researcher as a collaborator and submit a proposal for funding. Since VA has an intramural research program and does not award funds to non-VA scientists, such collaboration could allow for the submission of a research proposal to VA's investigator-initiated Merit Review Program in the Medical Research Service for possible merit-based funding. The Tulane investigators have indicated to VA officials that they intend to do this.

Our commitment to civilian and Federal researchers and to Gulf War veterans is to the support and funding of high quality research, which is best assured when all decisions on research funding are based on a process of rigorous, competitive, and independent peer review of all research proposals.

Sincerely,

Dr. Sue Bailey

JACK METCALF
2d District, Washington

COMMITTEE ON TRANSPORTATION AND INFRASTRUCTURE
SUBCOMMITTEES:
AVIATION
GROUND TRANSPORTATION

COMMITTEE ON SCIENCE
SUBCOMMITTEES:
ENERGY AND ENVIRONMENT

Congress of the United States
House of Representatives
Washington, DC 20515–4702

COMMITTEE ON BANKING AND FINANCIAL SERVICES
SUBCOMMITTEES:
HOUSING
FINANCIAL INSTITUTIONS
DOMESTIC AND INTERNATIONAL MONETARY POLICY

CHAIR, REPUBLICAN HOUSING OPPORTUNITY CAUCUS

REPUBLICAN POLICY COMMITTEE

September 27, 1999

The Honorable William S. Cohen
Secretary of Defense
The Pentagon
Washington, DC 20301-1010

Dear Secretary Cohen:

I was deeply disturbed by the response I received from Dr. Sue Baily regarding my letter to you dated May 13, 1999. I had requested that the Department of Defense (DOD) reconsider its answer to the General Accounting Office (GAO) in regards to their investigative report (GAO/NSIAD-99-5, "GULF WAR ILLNESSES: Questions About the Presence of Squalene Antibodies in Veterans Can Be Resolved," dated March 29, 1999) on the presence of squalene antibodies in some sick Gulf War-era veterans. Unfortunately, her letter of refusal only raised additional concerns about DOD's unwillingness to aggressively pursue answers for those suffering from Gulf War Illnesses.

One of the things most troubling to me over the past months, is the misinformation that DOD continues to provide publicly regarding this issue. The Tulane study demonstrates that ill Gulf War-era veterans have statistically distinct antibody levels to squalene when compared to other population groups. Various sources within DOD continue to assure the public and military members that squalene is naturally occurring in the human body and is found in over-the-counter items. Are you alleging that those who use these over-the-counter products containing squalene have similar antibody levels to sick Gulf War-era veterans being tested? If so, on what evidence are you basing your conclusion? How can we know unless we have an assay that is reliable? Is it not disingenuous for DOD to make such statements while avoiding the significant research it has done and continues to pursue in the area of adjuvant and vaccine development, and the potential use of squalene as an adjuvant component?

The recommendations of GAO are based on the sound belief that the first step in determining the significance of the Tulane results is to review the assay being used to produce the finding. The assay being used at Tulane is a variant of the common Western blot assay used routinely by the scientific community. If it is validated, then the work can begin to discover the clinical significance for those who are suffering. DOD has the scientists and resources to conduct a timely review that is inexpensive, expands on the research already conducted, and responds to the veterans who have waited over seven years for answers.

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While expressing assurance that DOD did not use adjuvants containing squalene during the Gulf War, Dr. Bailey closes her letter by encouraging the Tulane scientists to submit a research proposal. Why on the one hand has DOD been absolutely unyielding in their refusal to cooperate with GAO recommendations (for the DOD to conduct its own research designed to replicate or dispute the findings), while on the other, encouraging a formal research proposal on the very study we have repeatedly asked DOD to review?

In light of these events, I am requesting a complete review of DOD's work on squalene to date. Surely the DOD's research on experimental vaccines using adjuvant formulations containing squalene has provided data and meaningful insight regarding the consequences of their use. What research has been done by your department to assess the adverse health effects of these adjuvants? Did the trials include an 'adjuvant-only' test group to provide data regarding its safety? I am asking that you provide a clear picture for Congress and the public, of your work regarding adjuvant formulations so that rumor can be dispelled and replaced with fact.

Once again, because of your department's years of research in this area, I ask that you reconsider and proceed with the GAO recommendations. Your current position of waiting for the completion of the peer review and publication process does not recognize the vast amount of research that the DOD has already accomplished regarding adjuvant formulations containing squalene. The men and women who served honorably and are suffering from Gulf War Illnesses deserve truthful answers and *immediate action.*

I look forward to your personal reply.

Sincerely,

Jack Metcalf

Title VI
Report of the Comm on Appropriations 280

H.R. 2561
Dept of Defense Appropriations
Bill 2000

mittees by no later than January 31, 2000 on actions taken in the military health system to establish a systematic program for early detection and prevention of cervical cancer using the most modern and up to date screening methods.

GULF WAR ILLNESS

The Committee concurs with the findings of a recent GAO report on squalene antibodies and is concerned by the Department's reluctance to test for squalene antibodies since squalene is a potential contributing factor in illnesses of veterans of the Persian Gulf War. The Secretary of Defense is directed to develop and/or validate the assay to test for the presence of squalene antibodies. A report detailing the proposals to carry out this requirement shall be submitted to the Committee by January 1, 2000.

COMPUTER BASED MODELING IN HEALTH CARE

The Committee believes that computer based modeling and simulation capabilities may assist military health planners to assess the cost, access and quality impacts of reengineering delivery processes, delivery of protocols, and insertion of technology before committing vital resources. The Committee urges the Department to consider these management tools.

CHEMICAL AGENTS AND MUNITIONS DESTRUCTION, ARMY

Fiscal year 1999 appropriation	$780,150,000
Fiscal year 2000 budget request	1,169,000,000
Committee recommendation	781,000,000
Change from budget request	−388,000,000

COMMITTEE RECOMMENDATIONS

PROGRAM REDUCTIONS

The Army requested $1,169,000,000 for Chemical Agents and Munitions Destruction, Army. The Committee recommends $781,000,000, a decrease of $388,000,000. Of the decrease, $4,500,000 is taken with prejudice against program management consultants. Of the funds available, $75,303,000 shall be transferred to the Federal Emergency Preparedness Program to provide off-post emergency response and preparedness assistance to the communities surrounding the eight continental United States chemical storage and disposal sites.

The Chemical Agents and Munitions Destruction Program, Army mission is to safely destroy all U.S. chemical warfare munitions and related materiel while ensuring maximum protection of the public, personnel involved in the destruction effort, and the environment. The Committee commends the Army for its efforts in destroying chemical munitions in a safe manner. As of March 17, 1999, over 13.5 percent, or 4,259 tons, of the stockpile has been destroyed. Currently there are two sites operational and five sites in the design phase. Despite the fact that two additional sites are on hold until completion of the Assembled Chemical Weapons Assessment Demonstration, the Committee is hopeful that the U.S. will meet the deadline of April 2007 for the destruction of chemical munitions as called for by the Chemical Weapons Convention.

Signed into law
10/25/99

THE SECRETARY OF DEFENSE
WASHINGTON, DC 20301

NOV 03 1999

NOV 3 1999

Honorable Jack Metcalf
House of Representatives
Washington, DC 20515-4702

Dear Jack:

Thank you for your letter on the Department's position regarding the U.S. General Accounting Office report "Gulf War Illnesses: Questions about the Presence of Squalene Antibodies in Veterans Can be Resolved" (GAO/NSIAD-99-5).

We share your concern about troubling misinformation on this issue. The Department's position has been consistent and remains unchanged. Squalene was not used as an adjuvant in the anthrax vaccine. DoD gave no vaccines with squalene-containing adjuvants to U.S. troops deploying to the Gulf War. The Food and Drug Administration has verified that none of the vaccines used during the Gulf War contained squalene as an adjuvant. DoD contracted with an independent laboratory that verified that the anthrax vaccine does not contain squalene.

We asked the Tulane investigators to submit an application for research funding to validate their testing, but they did not. Our commitment to non-Government and Federal researchers and to Gulf War veterans is to support and fund research on potential causes of illnesses in Gulf War veterans. DoD is interested in looking at whether illnesses in service members are associated with antibodies to squalene. To do this, we need a scientifically proven test for squalene antibodies to assess whether Gulf War veterans have antibodies to squalene, hence our reason for pursuing additional research. In response to a DoD solicitation for research on illnesses among Gulf War veterans, a DoD investigator who is a nationally recognized expert in antibodies to cholesterol and other lipids, has been funded to pursue a study to determine the feasibility of developing a test for antibodies to squalene.

To date, the Tulane investigators have not succeeded in publishing their work in the medical literature. A draft of the Tulane paper was provided to the Research Working Group (RWG) of the interagency Persian Gulf Veterans' Coordinating Board. I have asked the RWG to provide you with a copy of its review of the draft Tulane paper. The review will contain the additional information on squalene that you requested.

Sincerely,

Title: Antibodies to Squalene
Project #: DoD-100
Agency: DoD
Study Location: Walter Reed Army Institute of Research (WRAIR), Forest Glen, MD
Project Status: Ongoing
Principal Investigator: Colonel Carl Alving, MD
Start Date: 1999
Completion Date: 2001
Phone: 301-319-9611

OVERALL PROJECT OBJECTIVE: Establish an effective means of testing for antibodies to squalene and determination of whether such antibodies are present in the blood of sick Gulf War veterans.

SPECIFIC AIMS: See objective.

METHODOLOGY: Clinical (immunologic) research.

EXPECTED PRODUCTS (MILESTONES): Establishment of appropriate testing method(s) for squalene antibodies and determination of whether such antibodies are present in a sample of ill Gulf War veterans.

STATUS/RESULTS TO DATE: The ELISA assay development is complete and control monoclonal antibodies have been successfully developed.

PUBLICATIONS: none to date

NOTES:

1. Colonel Alving submitted this proposal to the U.S. Army Medical Research and Materiel Command (USAMRMC) in FY99 under the Broad Agency Announcement (BAA) for Gulf War Illnesses Research projects.

2. An independent scientific peer review of Colonel Alving's proposal recommended that initial studies be limited to that part of the proposal directed toward induction of squalene antibodies. The peer review panel stated, "If antibodies to squalene cannot be induced, the subsequent studies proposed should not be initiated." The panel's final recommendation was, "...that only the first year of the proposal be funded until more information is provided on the experimental design and more importantly on whether or not antibodies to squalene exist."

3. The USAMRMC's Military Operational Medicine Research Program provided adequate funding to WRAIR to accomplish this objective. The funded project comprises five specific tasks to be completed by the end of FY00:

a. Develop and test ELISA assay for antibodies to squalene.

b. Evaluate and develop other assays for antibodies to squalene.

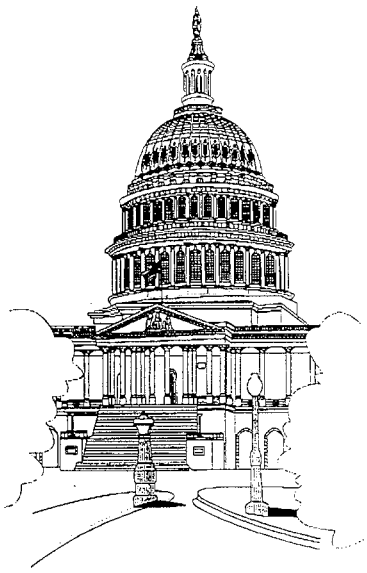
c. Develop a positive control antibody to squalene.

d. Large scale production of positive control antibody to squalene for use in assays.

e. Test normal human serum for antibodies to squalene by ELISA and other methods.

REPORT TO CONGRESS

GULF WAR ILLNESS



Development and Validation of an Assay to Test for the Presence of Squalene Antibodies

Executive Summary

This Report has been prepared in response to a requirement of the 106th Congress, House of Representatives, Report 106-244, 2000 Department of Defense Appropriations Bill:

> The Committee concurs with the findings of a recent GAO report on squalene antibodies and is concerned by the Department's reluctance to test for squalene antibodies since squalene is a potential contributing factor in illnesses of veterans of the Persian Gulf War. The Secretary of Defense is directed to develop and/or validate the assay to test for the presence of squalene antibodies. A report detailing the proposals to carry out this requirement shall be submitted to the Committee by January 1, 2000.

A May 1999 *Vanity Fair* article, "The Pentagon's Toxic Secret," alleged that the Department of Defense possibly used "an illicit and secret anthrax vaccine" on its own soldiers.[31] According to a Vanity Fair news release, "the licensed formula for...anthrax vaccine may have been altered, without formal FDA approval, to contain an experimental, and potentially dangerous, additive," squalene, that reportedly "causes incurable diseases in lab animals and may be the cause of some cases of Gulf War syndrome." The *Vanity Fair* article went on to suggest that the modified anthrax vaccine "may be part of the stockpile now being administered in the wake of the DoD's December 1997 decision to immunize 2.4 million people in the armed services against anthrax." A NewsWatch Associate editor presented an opposing review of the allegations entitled "Vanity Scare" in May 1999.[32]

On March 29, 1999, Congressman Jack Metcalf announced the release of a General Accounting Office (GAO) report, which he had requested, regarding squalene antibodies in veterans suffering from Gulf War illnesses. The GAO Report, "Gulf War Illnesses: Questions about the Presence of Squalene Antibodies in Veterans Can be Resolved" (GAO/NSIAD-99-5) recommended that DoD "conduct research designed to replicate or dispute the unpublished independent research results that revealed the presence of squalene antibodies in the blood of ill Gulf War-era veterans."[33]

In its investigations of illnesses among Gulf War veterans, the Senate Special Investigations Unit (SIU) found no credible information indicating that vaccines used during the Gulf War contained squalene.[38] In its report, the SIU stated that according to the Food and Drug Administration (FDA), squalene can be contained in a vaccine due to two different processes: 1) as an adjuvant, which is an agent to enhance the immune response; or 2) in minute quantities in vaccines manufactured using eggs, since eggs are rich in squalene and cholesterol. The FDA verified that none of the vaccines used during the Gulf War contained squalene as an adjuvant.

To investigate the squalene hypothesis, a scientifically proven test for squalene antibodies is needed to assess whether Gulf War veterans have antibodies to squalene. In response to a DoD solicitation for research on illnesses among Gulf War veterans, a DoD investigator and nationally recognized expert on antibodies to cholesterol and other lipids submitted a research proposal to determine the feasibility of developing a test for antibodies to squalene.

The funded research project to determine whether antibodies to squalene exist has five main objectives:

1) Development and validation of an ELISA assay for antibodies to squalene.
2) Evaluation and potential development of other assays for antibodies to squalene.

3) Development of a positive control antibody to squalene.
4) Production of the positive control antibody to squalene for use in the assays.
5) Testing of normal human serum for antibodies to squalene by ELISA and other methods.

The DoD funded study should provide adequate scientific evidence to resolve the issue of whether squalene antibodies exist and can be detected in human serum.

Background

Squalene is a relatively simple, linear hydrocarbon. It is a naturally occurring molecule in the human metabolic process that synthesizes cholesterol.[1] Squalene is present in human sebum and cell wall structures. Squalene is also a component of shark liver oil, some vegetable oils, and plant and animal cell membranes.[2] It is licensed by the FDA as a dietary supplement in the United States and is listed in the *Physicians' Desk Reference*. Squalene is used commercially in the cosmetic industry and in sunscreen products.[3]

Epidemiological studies of breast and pancreatic cancer in several Mediterranean populations have demonstrated that increased dietary intake of olive oil is associated with a small decreased risk or no increased risk of cancer, despite a higher proportion of overall lipid intake. Experimental animal model studies of high dietary fat and cancer also indicate that olive oil has either no effect or a protective effect on the prevention of a variety of chemically induced tumors. As a working hypothesis, it is proposed that the high squalene content of olive oil, as compared to other human foods, is a major factor in the cancer risk-reducing effect of olive oil. Experiments in vitro and in animal models suggest a tumor-inhibiting role for squalene.[4] In addition, studies using squalene in combination with low-dose pravastatin have demonstrated combination therapy significantly reduces total cholesterol and LDL cholesterol and increases HDL cholesterol to a greater extent than either drug alone.[5]

Squalene is one of several components of adjuvant formulations in a variety of vaccines.[6] One common formulation is MF59. MF59 is a safe, practical, and potent adjuvant for use with human vaccines.[7] Toxicology studies in animal models and Phase I-III studies in humans have demonstrated the safety of MF59 with HSV, HIV, and influenza vaccines.[7-17] Hilhers, et al, concluded that reactogenicity and stability but not adjuvanticity of synthetic sulfolipo-polysaccharide/squalane/water formulations depended on the molecular weight of synthetic sulfolipo-polysaccharide and that synthetic sulfolipo-cyclodextrin/squalane/water is a promising non-mineral oil adjuvant as it combines strong adjuvanticity (i.e. better than the mineral oil-based adjuvant presently applied) with low reactogenicity and good stability.[18]

However, Lorentzen has reported that the cholesterol precursor squalene (C30H50), through nonspecific activation of the immune system, can precipitate arthritis in rats. Using arthritis-prone rat strains to search for disease-triggering factors among molecules which initially induce innate defense reactions rather than specific immune responses, Lorentzen reported on the potential for endogenous lipids to precipitate arthritis.[19] In addition, there is evidence that in some instances squalene has a negative effect on the nervous system.[20-21]

Pamela B. Asa, Ph.D., an unaffiliated molecular biologist from Memphis, Tennessee and Yan Cao, M.D. and Robert F. Garry, Ph.D., from Tulane University, New Orleans, Louisiana have theorized that illnesses afflicting veterans of the Gulf War are an atypical connective tissue disease (an autoimmune disease) resulting from use of the vaccine adjuvant, squalene.[22-23] These investigators have reportedly developed an immunoassay for detecting anti-squalene antibodies and used the assay to test blood serum samples from various patient and control groups.

To investigate this hypothesis, DoD has funded a scientific program which will answer several major questions. Initially, the research staff will determine if antibodies to squalene exist and if an assay can be developed to detect and quantify these antibodies. In addition, an animal model will be used to induce anti-squalene antibodies to use as positive controls to characterize anti-squalene antibodies in

humans. If a positive antibody response to squalene can be induced in mice, then normal human serum can be tested for possible antibodies to squalene. Next, the research program will focus on qualitative detection of squalene and development of a chemical assay. Finally, the research program will examine the biological implications of antibodies to squalene.

Discussion

Pamela B. Asa, who has worked in the area of rheumatology and silicone-gel breast implants, presented a theory in 1995 of "human adjuvant disease" and its possible link to Persian Gulf War (PGW) Veterans' Illnesses. She theorized that silicone adjuvant (an agent added to a vaccine to increase antigenic response) was responsible for PGW veterans developing "human adjuvant disease."[24] A scientific review prepared by an independent non-governmental medical expert on September 13, 1995 of Dr. Asa's "Report on Gulf War Syndrome" found the basic hypothesis and supporting evidence presented was based on a series of erroneous assumptions and unsupported conjectures.[25] A similar review by the Medical, Chemical and Biological Defense Research Program found the basic hypothesis and supporting evidence presented by Dr. Asa were flawed or inaccurate.[26] Available information also strongly argues against Dr. Asa's hypothesis:

> All vaccines used during the Gulf War have a long history of safety and all, except BotTox that was used under an Investigational New Drug (IND), were licensed by the FDA at the time of the Gulf War.
>
> Since the standard immunization series is given to individuals in basic and advanced training, only a relatively small number of additional vaccines were given during deployment to the Persian Gulf, and the previous use of these vaccines has not resulted in problems similar to those reported by GW veterans.
>
> All vaccine lots are individually licensed for safety and efficacy. The vaccines used, therefore, are unlikely to be contaminated or of low quality.
>
> The only adjuvant used in the vaccines given to Gulf War personnel was alum. Alum is an FDA-approved adjuvant with a long history of safety. It has been given to millions of people worldwide without significant problems. No experimental adjuvants were used by the military.
>
> There are no reports of alum causing human adjuvant disease or any other chronic disease.
>
> There are no reports of chronic inflammatory responses at the sites of immunization with vaccines containing alum as would be expected if human adjuvant disease were to occur.
>
> Several recent studies have failed to show any association between silicone-gel implants and increased incidence of connective tissue disease. There is little supporting evidence, other than anecdotal reports, that silicone-gel implants cause an increase in connective tissue diseases or human adjuvant disease.

Dr. Asa's current work focuses on the presence of antibodies to squalene in a cohort of 142 Gulf War-era veterans or military employees. She theorizes that "Gulf War Syndrome" manifests a spectrum of signs and symptoms similar to that of other atypical connective tissue diseases and that most "Gulf War Syndrome" patients have serum antibodies to squalene, an immunological adjuvant. The study protocol attributes the hypotheses to findings in one (1) patient from a NIH-sponsored trial using squalene as an adjuvant.[22] The findings of the current unpublished work apparently originate from samples collected under this protocol. It is unknown if informed consent was obtained from individuals submitting samples for testing or if an Institutional Review Board (IRB) reviewed and approved the research protocol. Review of the draft manuscript indicates the basic hypothesis and supporting evidence presented as flawed or inaccurate. The findings from the study must be interpreted with caution as flawed methodology including biased sample selection and potential cofounders weaken any potential association. The following information also strongly argues against the current hypothesis:

> If in fact antibodies to squalene are present in Gulf War veterans, the clinical significance of finding these antibodies in humans is unknown. Squalene is normally present in humans as part of the body's production of

cholesterol. In addition, it is found in human sebum (skin oils) and plant and animal cell membranes. Antibodies to cholesterol in humans are common.

There may be alternative explanations for the reported laboratory findings, including: detection of naturally occurring squalene; cross-reaction with compounds similar to squalene; elevated levels of squalene due to a known or unknown disease process causing human illnesses, or; laboratory error or contaminant.

If in fact anti-squalene antibodies are present in the blood of Gulf War-era veterans, this is not sufficient to establish an association of squalene or squalene antibodies with any illness(es) among Gulf War veterans.

The assay for anti-squalene antibodies, which independent researchers at Tulane University developed, has not been validated at other laboratories nor have their findings been subjected to minimal peer review through publication in the scientific literature.

The only adjuvant used in the vaccines given to Gulf War personnel was alum. Alum is an FDA-approved adjuvant with a long history of safety. It has been given to millions of people worldwide without significant problems. No experimental adjuvants were used by the military.

The anthrax vaccine given to service members during the Gulf War and subsequently did not and does not contain squalene.

The Army Surgeon General has verified that the anthrax vaccine was never produced at any alternate production facilities in the U.S. during the Gulf War, and anthrax vaccine production at the Michigan Biologic Products Institute (MBPI, now BioPort) never contained squalene. Stanford Research Institute, International has recently completed verification testing for squalene on 6 lots of anthrax vaccine and verified that no squalene was detectable in any of the vials.

There are no data demonstrating increased rates of autoantibodies in ill Gulf War veterans.

Unfortunately, we cannot be sure that the theorists actually detected antibodies to a synthetic squalene adjuvant in the veterans they tested. They reportedly used a variation of a previously described assay.[27] This technique was used to claim findings of the first evidence from a blinded study of the existence of a laboratory marker that correlates with the severity of local and systemic complications in silicone breast implant recipients. The assay in question detects antibodies, not to silicone, but to a synthetic polymer whose characteristics have not been fully described. In subsequent letters to the editor, many noted the methodological flaws in the study, argued that since the antibody is not against silicone, there was no reason to suppose the implants had anything to do with the symptoms or antipolymer antibody assay test results, and noted that the investigators had reported similar high seroactivity in fibromyalgia patients.[28] A Committee named by the Institute of Medicine (IOM) recently reported that a careful study of all the evidence indicates that women with silicone breast implants are no more likely to develop chronic disease than women without the implants. The IOM Committee did not address antipolymer antibodies; however, they stated that "The clinical significance of a recently described antipolymer antibody test is unclear, although the polymer in question is not silicone or silicon containing, and it is extremely unlikely that it measures an antisilicone antibody."[29]

Dr. Garry and Tulane University reportedly received a U.S. patent in 1997 for an assay that could detect antibodies to polymers, of which squalene is one. In a letter from Dr. Garry to DoD, Re: Anti-Squalene Antibodies, dated May 7, 1999, Dr. Garry informed DoD that Tulane University Medical Center had applied for a patent on the use of anti-squalene antibodies in assessing Gulf War Syndrome. Dr. Garry also informed DoD that Tulane was the sole owner of the intellectual property provided in the letter of May 7, and that DoD should share the data only with those who have a specific need to know. In this letter, Dr. Garry reviewed the specifics of the anti-squalene antibody assay, or ASA Assay, that measures the binding of serum immunoglobulins to squalene.

The Office of the Army Surgeon General (OTSG) requested an update in early May 1999 on investigations, tests, and projects to investigate allegations regarding squalene in the anthrax vaccine and plans for developing an assay for squalene antibodies.[30] In the update, the Army stated that all lots of the anthrax vaccine released by DoD would be tested and that current testing to date by Stanford Research Institute, International confirmed that no squalene was detectable in any of the vials. The FDA is doing additional testing. Dr. Garry provided the manuscript outlining the details of his proposed assay to OTSG for review. It was the opinion of COL Alving and Dr. Matyas that there were "dozens of important technical and theoretical flaws" in the assay-many described by COL Alving as "fatal flaws." Dr. Garry had informed COL Alving and Dr. Matyas that, "even in the absence of peer-reviewed scientific validation, the patent rights to the technology for measuring antibodies to squalene had been exclusively licensed by Tulane University for commercial development by a company called, Autoimmune Technologies, L.L.C." Dr. Garry was unaware of the scientific literature that exists on antibodies to cholesterol. When informed of the antibodies to cholesterol by COL Alving, Dr. Garry "agreed that the purported antibodies that he observed might well represent antibodies that react with cholesterol."

Excerpts of the GAO report entitled, "Gulf War Illnesses: Questions about the Presence of Squalene Antibodies in Veterans Can be Resolved" stated that independent researchers had developed a test based on a Western blot assay and had detected antibodies to squalene in the blood of sick Gulf War veterans. If the description of the test described in the GAO report is accurate, there are some technical points that would seem to invalidate such a test:

> Squalene is a non-charged long chain hydrocarbon that would not be expected to migrate on a gel such as required in a Western blot assay.
>
> Because squalene lacks charge, it would not be expected to transfer to nitrocellulose as is done in a Western blot assay.

On March 29, 1999, Congressman Jack Metcalf (Washington) announced the release of a GAO report, which he had requested, regarding squalene antibodies in veterans suffering from Gulf War illnesses. The GAO Report, "Gulf War Illnesses: Questions about the Presence of Squalene Antibodies in Veterans Can be Resolved" (GAO/NSIAD-99-5) recommended that DoD "conduct research designed to replicate or dispute the independent research results that revealed the presence of squalene antibodies in the blood of ill Gulf War-era veterans."[32] The GAO did not comment on the ethical conduct of the research including a requirement for informed consent and IRB review of the protocol. The GAO did note that Chiron and Ribi ImmunoChem reported that their squalene adjuvant formulation had been tested on over 9,000 and 1,000 human subjects, respectively.

The clinical significance of finding antibodies to squalene is unknown. Squalene is normally present in humans as part of the body's production of cholesterol. It is found in human sebum (skin oils) and plant and animal cell membranes. The scientific work that has been done on squalene's role in human health and disease notes the positive effects of dietary squalene on cancer prevention and cholesterol regulation and the safety and efficacy of squalene as a vaccine adjuvant. There may be alternative explanations for the reported laboratory findings, including: detection of antibodies to cholesterol;[34-37] detection of antibodies to naturally occurring squalene; cross-reaction with compounds similar to squalene; elevated levels of squalene due to a known or unknown disease process causing human illnesses, or; laboratory error or contaminant.

The assay for anti-squalene antibodies developed by independent researchers at Tulane University has not been minimally validated through publication in the scientific literature. The investigators have

reportedly submitted a manuscript to a peer-reviewed medical journal; to date, however, this effort apparently has not been successful.

Since the Gulf War, squalene has been a component of vaccines undergoing testing by the Walter Reed Army Institute of Research (WRAIR). Volunteers received the vaccines in well-controlled studies that followed FDA regulations. Squalene is one of several components of the adjuvants found in each of two vaccine products undergoing testing by WRAIR. Pharmaceutical grade squalene is used to produce the oil emulsion used in these vaccine products. The exact compositions of the adjuvant in these vaccines are proprietary and belong to DoD Cooperative Research and Development Agreement (CRDA) partners. Development, evaluation, and FDA approval for the use of these adjuvant systems has been conducted by DoD CRDA partners and WRAIR. The two vaccines are investigational products for the prevention of malaria and human immunodeficiency virus (HIV) infection. Information on the study on the HIV vaccine has not yet been published and is considered proprietary information. Information on the study involving the malaria vaccine has been published in the scientific literature.[39]

Prior to its use in humans, the vaccines containing the emulsion underwent extensive FDA-mandated Good Laboratory Practices repeat dose toxicology studies involving rodents, rabbits, guinea pigs and nonhuman primates. The details of these studies (four volumes) were filed with the FDA as part of the IND application. The studies revealed anticipated inflammatory responses surrounding the site of injection. No gross changes were observed. No laboratory abnormalities were found.

Conclusion

Allegations of an ongoing conspiracy by the media and others is troubling. Squalene is not a foreign substance. It is normally present in the human body in large quantities because it is a precursor to the biosynthesis of cholesterol in the liver. The DoD funded study should provide adequate scientific evidence to resolve the issue of whether squalene antibodies exist and if they can be detected in human serum. Since squalene is being used as an adjuvant in some newer generation vaccines, this question becomes of interest not only to the military but also to the general public. Previously, these investigators were able to demonstrate antibodies to cholesterol. Squalene may not be immunogenic by itself, but under certain circumstances antibodies to the compound may arise. Although antibodies to cholesterol and possibly squalene occur naturally, this does not necessarily mean they have an adverse effect.

This research proposal was submitted in response to a competitive solicitation for proposals. The proposal was peer reviewed independent of the Department, by the American Institute of Biological Sciences, and received a high scientific merit score. Programmatic review was accomplished by the Department and the Research Working Group of the Persian Gulf Veterans Coordinating Board. Based on the results of this research, further studies can be pursued, if appropriate, to look at the existence of these antibodies in Gulf War veterans and their correlation to disease.

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CHAIR, REPUBLICAN HOUSING OPPORTUNITY CAUCUS

REPUBLICAN POLICY COMMITTEE

January 31, 2000

The Honorable William S. Cohen
Secretary of Defense
The Pentagon
Washington, DC 20301-1010

Dear Secretary Cohen:

We are writing to ask for an objective analysis of "Antibodies to Squalene in Gulf War Syndrome" - an article that has just been published in the February 2000 issue of *Experimental and Molecular Pathology.*

This peer-reviewed article found anti-squalene antibodies in a very high percentage of sick Gulf War-era veterans. As a bio-marker for the disease process involved in Gulf War Illnesses, the assay/blood test cited in the study could provide a vital diagnostic tool. We hope this will quickly lead to improved medical treatments for many who are suffering.

Many who have heard about this issue are anxious to understand the ramifications, especially those veterans and their families whose lives sadly have been directly affected. We certainly acknowledge the need for further research. However, that should not preclude a vigorous examination of the immediate benefits this study may provide medical practitioners treating those who suffer from Gulf War Illnesses.

The House passed version of the Fiscal Year 2000 Defense Appropriations Bill included report language instructing the Department of Defense to develop and/or validate the assay to test for the presence of squalene antibodies. This action was taken in response to DOD unwillingness to cooperate with the March 1999, General Accounting Office recommendation [NSIAD-99-5].
It reflected our firm belief that the integrity of the assay was the first step in finding answers.

Now that this study has been peer-reviewed and published, we need to take the next step and build on established science. An internal review by the same individuals within the DOD who were unwilling to cooperate for months does not constitute the kind of science that those who sacrificed for this nation deserve. Given the published article, it seems prudent to use the assay if it could help sick Gulf War era veterans. Do you agree?

We look forward to hearing from you by March 1, 2000. We thank you for your commitment and efforts on behalf of our Gulf War-era veterans.

Sincerely,

Jack Metcalf

Norm Dicks

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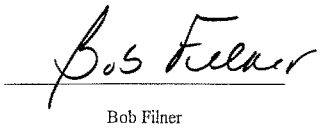
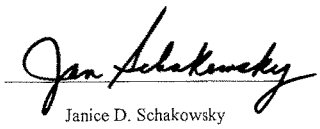
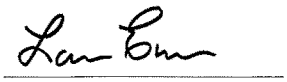
Walter B. Jones



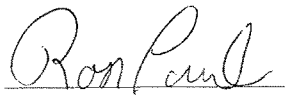
Bob Filner



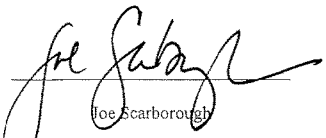
Janice D. Schakowsky



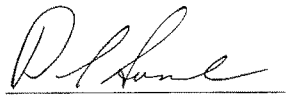
Lane Evans



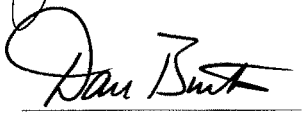
Ron Paul



Joe Scarborough



Bernard Sanders



Dan Burton

JACK METCALF
2D DISTRICT, WASHINGTON

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via facsimile 703-697-9080 - FINAL COPY

February 25, 2000

The Honorable William S. Cohen
Secretary of Defense
The Pentagon
Washington, DC 20301-1010

Dear Secretary Cohen:

I am exasperated and deeply disturbed by the Department of Defense's addition to its Anthrax Vaccination Inoculation Program (AVIP) website in the "Q & A" section under the heading "Production Issues" and the title "Accusations - Squalene."

On January 31, 2000, nine of my colleagues and I sent you a letter requesting an objective analysis of "Antibodies to Squalene in Gulf War Syndrome" - an article that had just been published in the February 2000 issue of *Experimental and Molecular Pathology* - a respected scientific peer-review journal. The letter represented our hope that DOD would seize the opportunity to do the kind of serious, scientific review that those who serve and sacrifice for our nation deserve.

Instead, a review of the AVIP website shows that DOD has chosen to do a hit-piece, dismissing "Antibodies to Squalene in Gulf War Syndrome" with the wildly expansive claim that "conclusions derived from the test results have **NO** scientific basis" (emphasis added). The marines, airmen, sailors and soldiers who access this site are not provided the courtesy of a rebuttal from the internationally respected scientist who developed the assay used in the research.

I am dismayed you would allow this posting to the website before you fully respond to the letter sent on January 31. DOD's action certainly reinforces the letter's concern regarding the inappropriateness of an internal review by the same individuals within DOD who have been unwilling to cooperate for nearly a year.

Additional information in this section is also troubling in its incompleteness. One section outlines "What does the U.S. Senate say about squalene?". Unfortunately, the site neglects to state that the 1998 conclusions made by the Senate Special Investigations Unit were made prior the GAO investigation, prior to the gathering of additional scientific data and more recently, findings in the House of Representatives.

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Page two--The Honorable William S. Cohen--February 25, 2000:

How can the DOD expect to regain the seriously eroded trust of its military personnel if misrepresentations posted on your official website are allowed to go unchallenged? Please take immediate action to remove the inappropriate and misleading response from DOD's information page, and do what is right - an objective analysis of the merits of this study.

Sincerely,

Jack Metcalf

Jack Metcalf
House of Representatives

cc: Representative Norm Dicks
Representative Walter Jones
Representative Bob Filner
Representative Janice Schakowsky
Representative Lane Evans
Representative Ron Paul
Representative Joe Scarborough
Representative Bernard Sanders
Representative Dan Burton

THE ASSISTANT SECRETARY OF DEFENSE

WASHINGTON, D. C. 20301-1200

FEB 28 REC'D

HEALTH AFFAIRS

Honorable Jack Metcalf
House of Representatives
Washington, DC 20515

FEB 24 2000

Dear Representative Metcalf:

Thank you for your letter asking for an objective analysis of Antibodies to Squalene in Gulf War Syndrome – an article published in the February 2000 issue of *Experimental and Molecular Pathology*. Prior to publication of the article, the Research Working Group (RWG) of the interagency Persian Gulf Veterans' Coordinating Board had objectively reviewed the work of Dr. Asa and her colleagues. We look forward to the scientific dialog and additional research that will now go forward as a result of long awaited publication of this data. I have enclosed the RWG review, our Report to Congress in response to the Fiscal Year 2000 Defense Appropriations Bill report language, and a review of the published article.

As you know, we have encouraged and awaited publication by these scientists ever since Dr. Asa first presented her theory on "human adjuvant disease" and its possible link to Persian Gulf War (PGW) veterans' illnesses. Prior to speculation about squalene, Dr. Asa theorized that silicone adjuvant (an agent added to a vaccine to increase antigenic response) was responsible for PGW veteran's developing "human adjuvant disease."

The Department published in the February 10, 1999 Commerce Business Daily a specific request for research proposals on "Interactions Of Drugs, Biologics And Chemicals In Service Members In Deployment Environments," supporting our research on illnesses among Gulf War veterans. This preceded the recommendation of the General Accounting Office to pursue research in this area. In response to this solicitation, a research proposal was submitted to develop and validate an assay to test for the presence of squalene antibodies. This proposal received a high independent scientific review merit score, was funded, and the research is ongoing.

We wholeheartedly agree that the integrity of the assay is the first step in finding answers. Our commitment to Gulf War veterans is to support and fund quality research. This is best assured when all decisions on research funding are based on a process of rigorous, competitive, and independent peer review of all research proposals. We are committed to responsible and aggressive pursuit of research that will further our understanding of illnesses among Gulf War veterans and prevent similar illnesses following future deployments.

Sincerely,

Dr. Sue Bailey

Enclosures:
As stated

Scientific Manuscript: "Antibodies to Squalene in Gulf War Syndrome"

The study, by Drs. P. B. Asa, Y. Cao and R. F. Garry, appeared in the February 2000 issue of Experimental and Molecular Pathology. The paper by Asa and colleagues presents data obtained by using an immunological assay that reportedly can detect previously unknown antibodies against squalene, a relatively simple, linear hydrocarbon that is a naturally occurring molecule in humans, animals and plants. Squalene is normally found in cell membranes in humans and is one of the building blocks for producing cholesterol.

Summary: Using this novel assay, the authors' report finding anti-squalene antibodies in a high percentage of "Gulf War Syndrome" patients. The antibody test developed at Tulane University Medical Center is called the Anti-Squalene Antibody Assay, or ASA Assay. Tulane has a patent pending on the ASA Assay, and Autoimmune Technologies LLC, a New Orleans biomedical company, has licensed the rights to the ASA Assay from Tulane.

The published research reportedly included both blinded and unblinded studies. In the blinded study, the ASA Assay was reportedly used to test blood samples from 56 individuals who were in active military service or who were civilian employees of the U.S. armed forces or their contractors during 1990-1991. Most, but not all, of the members of this group were reportedly deployed to the Persian Gulf theater of operations. The group comprised 38 deployed individuals who were ill, 12 deployed individuals who were healthy, and 6 non-deployed individuals who were ill. The results of the blinded study showed that 95% of the deployed sick individuals tested positive, none of the deployed healthy individuals tested positive, and 100% of the non-deployed sick individuals tested positive for anti-squalene antibodies.

In the unblinded study, the ASA Assay was used as a screening tool to gather further data. Blood samples from 86 additional individuals who were in active military service or who were civilian employees of the U.S. armed forces or their contractors during 1990-1991, including healthy individuals, were tested, and 69% of them tested positive. Because squalene is used as an ingredient in some cosmetics, 48 samples from blood banks were tested to see if the antibodies were present in a larger segment of the general population. Of these, 5% tested positive. To see if the antibodies were a marker for other autoimmune disease processes, 40 samples from patients with systemic lupus erythematosus were tested. Of these, 10% tested positive. Because patients with chronic fatigue syndrome have many symptoms similar to those of "Gulf War Syndrome" patients, 30 chronic fatigue patients were tested. Of these, 15% were positive.

The research also included a small adjunct study in which two individuals who had previously volunteered to participate in a vaccine trial in which squalene was an adjuvant in the vaccine were tested for the presence of anti-squalene antibodies. Both subjects tested positive. These two were the only patients in the research group who had a known exposure to squalene from vaccines.
The conclusion reached as a result of this research study is that most patients in the study groups who are ill with "Gulf War Syndrome" have serum antibodies to squalene while most other people do not. The clinical significance of the presence of the antibodies, however, is still not known, and while it is possible that the antibodies play a role in the disease process itself, the study does not explore the mechanisms involved in developing the antibodies.

Critical analysis: It is unknown if informed consent was obtained from individuals submitting samples for testing or if an Institutional Review Board (IRB) reviewed and approved the research protocol.

The authors claim to create a novel assay that detects antibodies to squalene. The authors however, do not use valid positive or negative controls. There are no positive controls (i.e., sera previously proven to contain antibodies to squalene) to validate the argument that the assay can detect antibodies to squalene. For positive controls, the authors cite only results obtained using this novel assay on two individuals reportedly vaccinated once and thrice with a squalene-containing adjuvant in a clinical trial sponsored by the National Institutes of Health. The authors provide no preimmunization results to demonstrate that the presumptive anti-squalene activity in the so-called positive controls was not present before immunization with the squalene adjuvant.

Fundamental to interpretation of novel assay data are negative controls. Such negative controls are critical to prove that the assay is not detecting artifacts (extraneous, cross-reacting substances). The authors have no negative control in which the human serum containing the presumed antibodies is omitted; there is no negative control in which the avidin-conjugated horse radish peroxidase is omitted; there is no negative specificity control for nonspecific binding of IgG, i.e., for normal IgG molecules sticking nonspecifically to squalene.

A further criticism of the paper is the authors use of only a single dilution of serum, rather than a series of dilutions. Without using this technique, there is a no way to obtain a titer, i.e., a quantitative measure of the degree of activity in the sample. The test results were scored at +++, ++, +, +/-, and -, raising the possibility that at high concentrations most normal sera might give a positive result; and the total absence of antibodies in a "normal" population must be regarded with some suspicion. If "squalene antibodies" or derivatives are associated with "Gulf War syndrome," one may expect titers to parallel severity of symptoms. The paper gives no evidence of this.

The assay by Asa and colleagues remains an unvalidated and unproven assay.

JACK METCALF
2D DISTRICT, WASHINGTON

COMMITTEE ON TRANSPORTATION AND INFRASTRUCTURE
SUBCOMMITTEES:
AVIATION
GROUND TRANSPORTATION

COMMITTEE ON SCIENCE
SUBCOMMITTEE:
ENERGY AND ENVIRONMENT

Congress of the United States
House of Representatives
Washington, DC 20515-4702

COMMITTEE ON BANKING AND FINANCIAL SERVICES
SUBCOMMITTEES:
HOUSING
FINANCIAL INSTITUTIONS
DOMESTIC AND INTERNATIONAL MONETARY POLICY

CHAIR, REPUBLICAN HOUSING OPPORTUNITY CAUCUS

REPUBLICAN POLICY COMMITTEE

via facsimile 703-697-9080

March 3, 2000

The Honorable William S. Cohen
Secretary of Defense
The Pentagon
Washington, DC 20301-1010

Dear Secretary Cohen:

Please intervene to halt the obfuscation campaign Department of Defense officials seem intent on conducting concerning the issues surrounding antibodies to squalene research. Monday, February 28, 2000, I received a response to the letter I had sent to you. Nine of my colleagues in the House of Representatives joined me to request that DOD do an objective analysis of "Antibodies to Squalene in Gulf War Syndrome" -- an article recently published in the February 2000 issue of *Experimental and Molecular Pathology.*

DOD's letter, authored by Dr. Sue Bailey, avoids providing Congress a clear and direct answer to our request. The following excerpts illustrate my concerns with DOD's official reply.

1. In paragraph one, Dr. Bailey states that she has enclosed the Research Working Group (RWG) review. She does not mention that the RWG reviewed an early draft of the study, provided to them as a professional courtesy. The text of the final peer-reviewed article contains some significant changes. Members of Congress asked for an objective analysis of the peer-reviewed article. It is difficult to understand why Dr. Bailey chose to include a review not based on the published scientific article, unless her goal was confusion rather than clarity.

2. Also provided as an attachment, and referenced in paragraph one, is a review of the published article. I was dismayed that Dr. Bailey would provide this brief summary with no indication of the author's name or professional credentials to conduct and provide such a review. My colleagues and I stated clearly, "An internal review by the same individuals within the DOD who were unwilling to cooperate for months does not constitute the kind of science that those who sacrificed for this nation deserve." A half-page critical analysis, anonymously written, is not an appropriate response to the congressional request.

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3. Dr. Bailey continues in paragraph two by making a reference to an early theory that is completely irrelevant to our request. Dr. Asa's early and *confidential* correspondence with DOD regarding potential cause was motivated by concern for those suffering from Gulf War Illnesses. DOD must encourage researchers to explore hypotheses rather than setting them up for public criticism, if we are going to solve the mystery of Gulf War Illnesses. The congressional inquiry's focus is the peer-reviewed study and the assay used to detect the antibodies. Dr. Bailey's reference is an unnecessary distraction from the facts.

4. Dr. Bailey's third paragraph attempts to portray DOD as proactive in developing and validating an assay to test for the presence of squalene antibodies prior to the GAO recommendations. Nothing could be further from the truth:

A. DOD's response to the GAO accused them of being "scientifically and fiscally irresponsible" for suggesting that DOD conduct research to dispute or validate the independent research findings. DOD's position was clear: until the peer-review and publication process by the private scientists was completed, it would not consider action that could provide answers to those suffering from Gulf War Illnesses. (GAO/NSIAD-99-5)

B. When DOD was interviewed by GAO during the investigation, its spokespersons acknowledged DOD had the know-how to develop such an assay and could have tested for squalene antibodies but did not.

C. When Dr. Bailey provided DOD's final comments to the GAO report, she stated, "Our position and the concerns expressed in our comments to the draft report have not changed." (DOD letter to the GAO dated May 28, 1999)

D. It was only after the U.S. House of Representatives took action and instructed DOD to cooperate with the GAO recommendations that Congress received notice from DOD of its funding of related research. This confirmatory research is being conducted by a DOD researcher. *(House of Representatives Report 106-244, Department of Defense Appropriations Bill, 2000)*

In light of these facts, it is disturbing that Dr. Bailey would construct paragraph four in such a way as to revise the sequence of events, and in doing so, misrepresent DOD's consistent position prior to legislative action.

In closing, Dr. Bailey states, "We are committed to responsible and aggressive pursuit of research that will further our understanding of illnesses among Gulf War veterans and prevent similar illnesses following future deployments." Unfortunately, something vital is missing from her statement: treatment and answers for those who are suffering. It is not acceptable to ask sick Gulf War-era veterans and their families to wait decades for endless research projects which do not generate help and treatment for those suffering. The consequences of this failed policy approach are all too clear to Congress, the American public, and especially the veterans exposed to and sickened by Agent Orange during the Vietnam War.

Our request to you on January 31, 2000 was straightforward and simple: determine if the assay used in the peer-reviewed, published study could be utilized as a diagnostic tool to help sick Gulf War era veterans. I would greatly appreciate your personal assistance to insure that DOD provide the objective analysis initially requested, including identification of those who are providing the analysis and their

professional credentials.

Thank you.

Jack Metcalf

Jack Metcalf
House of Representatives

cc: Representative Norm Dicks
Representative Walter Jones
Representative Bob Filner
Representative Janice Schakowsky
Representative Lane Evans
Representative Ron Paul
Representative Joe Scarborough
Representative Bernard Sanders
Representative Dan Burton

THE ASSISTANT SECRETARY OF DEFENSE
1200 DEFENSE PENTAGON
WASHINGTON, DC 20301-1200

HEALTH AFFAIRS

MAR 27 REC'D

Honorable Jack Metcalf
United States House of Representatives
Washington, DC 20515-4702

Dear Congressman Metcalf:

Thank you for your recent letters on the Anthrax Vaccine Immunization Program's website and on the information I provided to you as requested in your inquiry of January 31, 2000. To address your request for additional objective analysis of this article, I have asked the Armed Forces Epidemiological Board to convene a subcommittee of experts to review and critique this work. I will provide you with this critique and, as requested, the curricula vitae of the reviewers. In addition, the National Academy of Sciences, Institute of Medicine (IOM), is assessing the role squalene may play as a cause of illnesses among Gulf War veterans and reviewing the work of Dr. Asa and her colleagues. The IOM expects to publish a report in August of this year.

The Department has considered your comments and suggestions regarding the Anthrax Vaccine Immunization Program's website. On March 10, 2000, the portion of the website describing the antibody test developed by Dr. Asa and colleagues was modified to read as follows: "Whether or not this test has any clinical meaning will be settled by medical experts over time. For now, it is sufficient to recognize the conclusions of the authors: "It is important to note that our laboratory-based investigations do not establish that squalene was added as adjuvant to any vaccine used in military or other personnel who served in the Persian Gulf War era.""

Our commitment to Gulf War veterans is unwavering. All known, testable hypotheses concerning illnesses among Gulf War veterans have been or are being pursued through our program of basic science research. All decisions on research funding are based on a process of rigorous, competitive, and independent peer review. We are committed to responsible and aggressive pursuit of research that will further our understanding of illnesses among Gulf War veterans and prevent similar illnesses following future deployments.

Sincerely,

Dr. Sue Bailey

Experimental and Molecular Pathology 68, 196–198 (2000)
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LETTERS TO THE EDITOR

To the Editor:

A recent article in this journal by Asa *et al.* (2000) purports to measure serum antibodies to squalene. The paper fails to establish the validity of the test. The essential flaws involve selection of proper positive controls and proper negative controls, quantitative methods, and selection of study populations.

The authors hypothesize that antibodies are induced by "the adjuvancy of squalene," such that injection of squalene could elicit antibodies to squalene. One approach might be to inject squalene into an experimental animal to determine *first* whether the injection can induce the purported antibodies and *second* whether the assay can detect the induced antibodies. Antibodies induced by injection, if they exist, could then serve as a positive control for the unvalidated assay.

The assay describes no positive controls that actually validate the assertion of detecting antibodies to squalene. Such positive controls would consist of comparable serum samples demonstrated to contain anti-squalene antibodies after injection with squalene.

The authors assert that they have positive controls, in the form of two human subjects previously injected with a squalene-containing placebo during a clinical trial at the National Institutes of Health. However, the authors provide no preinjection results to establish that intentional injection of squalene led to antibodies to a substance already present in the body.

The assay also lacks elementary negative controls routinely run in enzyme-linked immunoassays. Such negative controls are required to prove that the assay is not detecting cross-reacting substances. In a new, unproven assay that claims to detect a novel antibody, one must prove specificity. There were no negative controls in which the human serum containing the presumed antibodies was omitted or in which the avidin-conjugated horseradish peroxidase was omitted. There is no evidence that the assay was not simply measuring other IgG molecules with nonspecific binding to squalene. This could be easily accomplished by substituting an oil molecule similar to squalene. An excellent negative control would be squalane, the fully hydrogenated form of squalene.

The unknown human serum samples were tested only at a single dilution (1:400). Most assays for naturally occurring antibodies, particularly antibodies to lipids, start at a higher concentration of serum, typically a dilution of 1:50. Thus, the method of Asa *et al.* could miss the presence of antibodies detectable at a higher concentration of serum. It is possible that normal blood donors could give positive results at a higher concentration of serum.

A further drawback of using only a single dilution of serum, rather than a series of dilutions, is that there is no way to obtain a quantitative measure of the degree of activity in the sample. Titers are routinely obtained when antibody levels are measured. The absence of quantitation in this assay weakens meaningful comparisons between unknown serum samples from subjects accrued in a nonrandom manner.

Figure 1, said to show "antisqualene antibody responses," is particularly flawed. In this figure, unspecified quantities of squalene were added as aqueous dilutions of 1:10, 1:100, 1:1000 and 1:10,000 for impregnation of nitrocellulose. No explanation is provided for how an oil such as squalene, not soluble in water, could be diluted in water by the published methods. Further, a washing solution containing polyoxyethylene sorbitan monolaurate could have detergent-like qualities that could remove squalene. Despite the extensive dilutions of the squalene, there is no evidence of a dilution curve (assessing each strip vertically), regardless of whether the antibody reactions were rated as 3+, 2+, or 1+. This suggests that nonspecific binding of serum immunoglobulin may have occurred.

The conclusions of Asa and colleagues, purporting to correlate anti-squalene with Gulf War illnesses, in our opinion, rely on circular logic. Positive results with an assay not previously validated to detect antibodies cannot be used as scientific proof that antibodies to the antigen exist in samples of unknowns. It is premature to proceed directly to testing

0014-4800/00

serum samples from healthy people and sick people before conducting the fundamental validation steps.

The critique offered here is not meant to imply that antibodies to squalene do not or cannot exist. As pointed out by the authors, extensive work demonstrates that antibodies to cholesterol, a molecule for which squalene serves as a precursor, are found in virtually all normal human sera. A recent report proposes that naturally occurring antibodies to cholesterol may serve a vital physiologic function in helping regulate low-density lipoprotein metabolism in humans (Alving and Wassef, 1999).

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Carl R. Alving

Walter Reed Army Institute of Research
Silver Spring, Maryland 20910

John D. Grabenstein

U.S. Army Medical Command
Falls Church, Virginia 22041

This letter is doi:10.1006/exmp.2000.2314

Reply

To the Editor:

Alving and Grabenstein declare that our methods "do not establish the validity of the test." They are mistaken and have made a number of false assumptions about our methods and about which experiments were and were not performed to validate the anti-squalene antibody (ASA) assay. We also strongly disagree that animal work must precede human studies.

Our study (1) is the first description of anti-squalene antibodies in humans. Replicating our results in an animal model may well be useful for studying the possible role of ASA in Gulf War Syndrome (GWS), but is not a prerequisite by any standards we (or the peer reviewers of our manuscript) are aware of for establishing the validity of an immunoassay. For example, it was not essential to demonstrate antinuclear antibodies (ANA) in animals to develop a useful ANA assay for human autoimmune disease. Moreover, there is no assurance that small animals or even primates would respond immunologically to a squalene challenge. Production of ASA may require coinjection with or coexposure to additional substances or an autoimmune process not readily reproduced in an animal model.

It would also be unethical to inject squalene, a substance that has a 25-year history of causing both autoimmune rheumatological disease and neurological disease (Lorentzen, 1999; Grajkowska *et al.*, 1999), into humans to see if we could raise antibodies to it.

The ASA assay, a variation on the well-characterized Western blot assay, was validated by standard approaches used in immunoassay development. Alving and Grabenstein assert that "the assay lacks negative controls." However, each of the "elementary" negative controls they suggested, as well as many other controls, was in fact performed. The descriptions of these simple tests were not included in our paper for brevity. Assays in which either human serum or avidin-conjugated horseradish peroxidase was omitted gave no reaction. It should be noted that the reagents we used are precisely the same stringently validated reagents used to detect human antibodies to human immunodeficiency virus in commercially available Western blot assays. Squalane, a molecule similar to squalene, also gave no reaction in this assay. Furthermore, preincubation of positive human sera with squalene (but not squalane or other oils) blocked the assay in a dose-dependent manner. Squalene did not block another immunoassay, the HIV Western blot, further confirming the validity of the ASA assay.

Alving and Grabenstein are incorrect in their assumption that "the samples were tested at only a single dilution." In the process of optimizing the ASA assay, samples were tested at varying dilutions between 1:25 and 1:4000. 1:400 was determined to be the optimal dilution.

We did not indicate that squalene was soluble in water. Squalene, like many oils, can be finely dispersed in water and diluted as indicated. Western blot-style immunoassays differ from other types of immunoassays. Titers are not routinely obtained in Western blot-style immunoassays. At lower serum dilutions, some normal donors do react on the

ASA assay. This is to be expected and does not change the conclusions stated in our paper in any way.

Alving and Grabenstein assert that "a washing solution containing polyoxyethylene sorbitan monolaurate could have detergent-like properties that could remove squalene." This speculation is directly refuted by the results we presented. The ASA assay is similar in format to Western immunoblotting, in which proteins are tightly bound to nitrocellulose strips simply by drying. A similar method was used to apply squalene to the nitrocellulose strips used in the ASA assay. For this molecule, as with proteins in Western blots and nucleic acids in Southern and Northern blots, hydrostatic and other interactions with nitrocellulose are strong enough to resist removal by a weak detergent.

It is extremely unlikely that our results can be explained by "nonspecific binding of serum immunoglobulin." If this were the case, then similar or higher percentages of healthy donors or autoimmune patients (many of whom were hypergammaglobulinemic) would have detectable binding of serum antibodies in the ASA assay compared with GWS patients (ASA *et al.*, 2000). As this was not observed, the use of sera from these appropriate control populations further validates the ASA assay.

The ASA assay was rigorously validated by standard immunological methods prior to testing of serum samples from healthy and sick individuals. Circular logic was not used, and we stand firmly by the conclusions of our manuscript.

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Pamela B. Asa
Yan Cao

Memphis, Tennessee

Robert F. Garry

Tulane Medical School
New Orleans, Louisiana 70112

This letter is doi:10.1006/exmp.2000.2315

Editorial Note

New findings require confirmation, within the bounds of comparability. This is as true for methodology as it is for the data produced from a particular study. This exchange of letters from the Office of the Surgeon General, United States Army, and the authors of "Antibodies to squalene in Gulf War Syndrome," *Exp. Mol. Pathol.* 68, 55–64 (2000), relates to methodology. Drs. Alving and Grabenstein offer no data against the conclusions of Asa *et al.*

The exchange will be judged by the scientific community on its merits, as all such matters should be. We point out only that Asa *et al.* are correct in their reply when they note that Western blot methods do not routinely measure relative titers, although some laboratories may report an intensity grade from the bands produced (e.g., 1+ to 4+).

The Editors

This note is doi:10.1006/exmp.2000.2316

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Results 1 to 1

Letter
Carl R. Alving, John D. Grabenstein
Experimental and Molecular Pathology, Vol. 68, No. 3, Jun 2000, pp. 196-197 (doi:10.1006/exmp.2000.2314)

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THE ASSISTANT SECRETARY OF DEFENSE
WASHINGTON, D. C. 20301-1200

HEALTH AFFAIRS

AUG 10 REC'D

AUG 2 2000

Honorable Jack Metcalf
House of Representatives
Washington, DC 20515-4702

Dear Representative Metcalf:

I am pleased to provide you with the objective analysis that you requested for the article "Antibodies to Squalene in Gulf War Syndrome," published in the February 2000 issue of *Experimental and Molecular Pathology*. The Armed Forces Epidemiological Board convened a subcommittee of experts to review and critique this article and the attached response was unanimously endorsed and approved by the Board.

I hope we have answered the questions raised in your letter. Thank you for your interest in the health of Gulf War veterans.

Sincerely,

J. Jarrett Clinton, MD, MPH
Acting Assistant Secretary

Attachment:
As stated

cc:
Special Assistant for Gulf War Illnesses

DEPARTMENT OF DEFENSE
ARMED FORCES EPIDEMIOLOGICAL BOARD
5109 LEESBURG PIKE
FALLS CHURCH VA 22041-3258

AFEB (15-1a) 00-6 11 July 2000

MEMORANDUM FOR THE ASSISTANT SECRETARY OF DEFENSE (HEALTH AFFAIRS)
THE SURGEON GENERAL, DEPARTMENT OF THE ARMY
THE SURGEON GENERAL, DEPARTMENT OF THE NAVY
THE SURGEON GENERAL, DEPARTMENT OF THE AIR FORCE

SUBJECT: Armed Forces Epidemiology Board (AFEB) Recommendations Regarding Review of the Paper, "Antibodies to Squalene in Gulf War Syndrome by P. B. Asa, Y. Cao and R. F. Garry."

1. The AFEB was tasked by the Department of Defense (Health Affairs) to conduct an objective analysis of the above paper following a request by Congressman Jack Metcalf to Health Affairs.

2. A Special Subcommittee was formed to review the paper. Results of the review and the paper were distributed to the rest of the Board prior to the AFEB meeting. The Subcommittee's findings were presented to the whole Board at the AFEB Meeting held 28-29 February 2000 at Fort Sam Houston, Texas. After discussions and several additional reviews, the report was finalized.

3. The AFEB has thoroughly reviewed the paper by Dr. Asa and colleagues who describe a laboratory test they feel may identify individuals ill with "Gulf War Syndrome." The following is a summary of the findings:

a. THE RESEARCH REPORTED IN THIS PAPER DOES NOT SUPPORT THIS CLAIM.

b. THE PAPER CONTAINS NUMEROUS SHORTCOMINGS, SEVERAL OF THEM SERIOUS, THAT COMBINE TO INVALIDATE THE AUTHORS' CONCLUSIONS.

c. IT REMAINS UNCLEAR IF THE ASSAY ACTUALLY MEASURES ANTIBODIES TO SQUALENE, AS THE AUTHORS ASSERT; THE ASSAY MAY MEASURE SOMETHING ELSE OR THEIR FINDINGS MAY BE A NON-SPECIFIC CHEMICAL REACTION.

AFEB (15-1a) 00-6 11 July 2000

SUBJECT: Armed Forces Epidemiology Board (AFEB) Recommendations Regarding Review of the Paper, "Antibodies to Squalene in Gulf War Syndrome by P. B. Asa, Y. Cao and R. F. Garry."

4. The Board unanimously endorses and approves the above findings and the enclosed report. Details of their findings can be found in the enclosed report.

FOR THE ARMED FORCES EPIDEMIOLOGICAL BOARD:

F. MARC LAFORCE, M.D.
AFEB President

BENEDICT M. DINIEGA
Colonel, USA, MC
AFEB Executive Secretary

3 Encls
1. Report
2. Tasking Letter
3. CVs

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REVIEW OF THE PAPER

ANTIBODIES TO SQUALENE IN GULF WAR SYNDROME
by PB Asa, YCao and RF Garry

published in
***Experimental and Molecular Pathology*, Volume 68, pp 55-64 (2000)**

A REPORT FROM
THE ARMED FORCES EPIDEMIOLOGICAL BOARD
JUNE 22, 2000

SUMMARY OF FINDINGS

The Armed Forces Epidemiological Board has thoroughly reviewed the paper by Dr. Asa and colleagues who describe a laboratory test they feel may identify persons ill with "Gulf War Syndrome." The AFEB has concluded unanimously that the research reported in this paper does not support this claim. The paper contains numerous shortcomings, several of them serious, that combine to invalidate the authors' conclusions. It remains unclear if the assay actually measures antibodies to squalene, as the authors assert; the assay may measure something else, or their findings may be a non-specific chemical reaction.

BACKGROUND

The Armed Forces Epidemiological Board (AFEB) was tasked by the Department of Defense (Health Affairs) to conduct an objective analysis of the above captioned paper by Asa *et al.* The tasking letter is enclosed.

A special subcommittee[1] of the AFEB was formed to initiate the task. The Special Subcommittee read the above captioned paper by Asa *et al.* The subcommittee fully discussed its impressions, questions and concerns, and developed a consensus document. The chair of the subcommittee then formally presented the subcommittee's findings to the entire AFEB[2] which had been supplied with the paper and the consensus document in advance of the meeting. After input from the entire AFEB, this final report is offered to the requester by the AFEB president.

FINDINGS

The AFEB reviewed the paper with great interest. However, the AFEB found the paper to contain a large number of scientific flaws, some of which are extremely grave. These flaws invalidate to an almost complete degree the conclusions regarding squalene and the implications that proceed from them. The major flaws include the following:

Controls: Despite assertions and disclaimers in the paper, there are no valid controls.

- For a valid positive control, one needs serum previously proven to contain antibodies to squalene; only this can validate that the assay can detect antibodies to squalene. What the authors use as and assert is a positive control are two sera from individuals reportedly vaccinated (either once or three times) with an NIH trial vaccine containing squalene. The authors provide no pre-vaccination data to demonstrate that the activity detected in their assay was not present before vaccination with a squalene adjuvant.
- Negative controls are essential to prove that the assay is not detecting something other than anti-squalene antibodies. Missing are controls which omit serum containing the presumed antibodies or which omit the avidin-conjugated horse radish peroxidase. Also missing is a negative specificity control to rule out non-specific binding of normal IgG molecules to squalene.

Blinding: It is unclear if the researchers were blind as to illness/wellness status of study participants.

- The paper asserts at several points that this is a blinded study, but it remains possible that the critical element of knowing the illness/wellness status or category may have been known, even if, as the paper states, "...The identities or exact number of samples from each category were not made available..."

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[1] S-Music, Chair; E Barrett-Connor, P Landrigan, Members; *curricula vitae* attached per written request of Congressman Metcalf to Defense Secretary Cohen, as "...objective analysis...including identification of those who are providing the analysis and their professional credentials."

[2] During the 30-31 May 2000 meeting of the AFEB at Ft. Detrick, MD.

- Thus, the authors' assertions, that they did not know which subjects had "Gulf War Syndrome" and which did not, are not convincing. If the authors knew which blood samples came from Gulf War veterans, this could have biased their interpretation of their test findings.

Specificity: Does the ASA Assay actually measure antibodies to squalene?

- In this type of blotting experiment, one normally demonstrates specificity of the reaction by blocking (or adsorbing) the antibody with the antigen (in solution). This is not demonstrated.
- Hence, it is not possible to know what the ASA assay detects. It is a Western-blot type assay, and is either positive (+) or negative (–). Since the paper describes it being used in only one dilution of patient serum (1:400), it seems the assay can determine only whether "something" was detectable or not, and this "something" is not presently definable.
- Antibodies to squalene, or to any other substance for that matter, should be detectable across a range of concentrations, so antibody assays are normally constructed to demonstrate this, the most common form today being an enzyme-linked immunoassay (ELISA). The actual level or concentration of antibody, ranging from undetectable to just detectable through high concentration, should have medical/biological correlations and implications, with some threshold point that correlates with the development of symptoms or disease.
- Nitrocellulose is a highly reactive substance that binds many materials. The paper does not show that the squalene deposited on the membrane is actually still there at the end of the assay. For example, one could imagine that squalene could "block" the nitrocellulose membrane long enough to protect the "dot" from the milk treatment and then be washed out, as polyoxyethylene sorbitan laurate is a detergent that could remove a lipid like squalene. This could leave a naked spot of nitrocellulose to react with some other protein.
- If this were a valid assay it should work with another substrate (other nylon membranes, like Immobilon).
- Given the relationship between squalene and cholesterol, do these sera react with cholesterol? The authors raise the question but don't answer it.
- Can one actually raise antibodies, deliberately, to squalene? It is a common component of cells and should be present in amounts that would swamp out any squalene-specific antibodies.

Dose response: None is apparent.

- In the figures of the Asa *et al* paper, there is no obvious dose response in relation to the amount of antigen (squalene) deposited on the nitrocellulose membrane.
- A dose-response should be seen with respect to antigen and antibody concentration; neither is shown.

CONCLUSIONS

In summary, the clear failure to provide positive controls and negative controls as well as unambiguous blinding, invalidates the authors' ability to argue for the meaningfulness of their test and any conclusions they might draw from these results. This is true even before one gets to the more technical issue of the specificity of the ASA assay.

Therefore, the AFEB has little confidence that the patent-pending ASA assay actually measures antibodies to squalene, though we cannot entirely eliminate this possibility.

Whatever the paper's flaws and since the AFEB cannot exclude the remote possibility that the authors have identified a laboratory means of distinguishing persons with possible Gulf War Syndrome (GWS) from all others, replicability becomes the major unresolved issue. The AFEB recognizes the difficulties inherent in defining a possible case of GWS since there is no standardized case definition. However, the AFEB feels that the symptom list in the Asa *et al* paper is a good potential starting point, and that, for example, cases might be selected from tertiary referral centers for GWS such as the one at Walter Reed, with controls from a civilian, non-exposed workforce. Therefore we recommend that a suitable test of replicability be done in cooperation with the authors and with attention to the following design elements:

- selection of participants - cases and control subjects - by an independent *ad hoc* body or committee, chaired by a tenured academic from a well-known medical research institution
- establishing clear *a priori* selection and exclusion criteria for cases and for controls
- serological testing done in a secure and absolutely blind manner with strict chain of custody rules and documentation in place
- a sufficient number of subjects to have statistical power to detect a true difference, if one exists, with 80% likelihood and with a 5% chance or less of finding a difference due to random chance alone.
- a study design with at least two arms – testing done as in the paper by the people who have licensed this patent-pending technique, versus testing done by one or more lipid laboratories using more standard antibody techniques such as enzyme-linked immunoassay to detect antilipid antigens

We wish to be clear that we are not discussing a study to validate whether the ASA assay can detect antibodies to squalene. Rather, we are trying to leap over this intermediate obstacle and get quickly and inexpensively to a more meaningful bottom line: does the ASA assay clearly, reliably and unequivocally distinguish people with GWS from all others, and, if so, with what specificity and sensitivity? Many caveats and qualifiers would have to be in place to assure meaningfulness, and the preceding bulleted list can (and probably should) be usefully expanded and further refined to help assure that any ensuing serological study be definitive.

The AFEB is extremely doubtful that the assay reported by Asa *et al* is a valid or accurate test for illness among Gulf War veterans. However in an effort to leave no stone unturned in evaluating veterans' complaints, the AFEB feels it may be worthwhile to repeat the study, using appropriate scientific methods as outlined above. This recommendation should definitely not be considered an endorsement of the paper by Asa *et al* that we have herewith reviewed.

OFFICE OF THE ASSISTANT SECRETARY OF DEFENSE
1200 DEFENSE PENTAGON
WASHINGTON, DC 20301-1200

HEALTH AFFAIRS

09 MAR

MEMORANDUM FOR EXECUTIVE SECRETARY, ARMED FORCES EPIDEMIOLOGICAL BOARD

SUBJECT: Objective Analysis of Article "Antibodies to Squalene in Gulf War Syndrome"

I request that the Armed Forces Epidemiological Board (AFEB) convene a subcommittee and review and provide OASD(HA) with an objective analysis of the attached article, "Antibodies to Squalene in Gulf War Syndrome" published in the February 2000 issue of *Experimental and Molecular Pathology*. Congressman Jack Metcalf requested this objective analysis. Congressman Metcalf would also like the curriculum vitas of the reviewers.

OASD(HA) will provide Congressman Metcalf with this critique and the curriculum vitas of the reviewers when complete. Please provide this review NLT 15 May 2000. To assist in this review, I have attached an extensive review of the work on squalene as a cause of illnesses among Gulf War veterans by the interagency Research Working Group of the Persian Gulf Veterans Coordinating Board prior to publication of the article and previous correspondence with Congressman Metcalf's office on this topic.

My point of contact is James R. Riddle, LtCol, USAF, BSC, (703) 681-1703, fax (703) 681-3655, or email james.riddle@ha.osd.mil.

John F. Mazzuchi

John F. Mazzuchi, Ph.D.
Deputy Assistant Secretary of Defense
Clinical and Program Policy

Attachments:
As Stated

CURRICULUM VITAE

I. PERSONAL DATA

A. Name: Stanley I. Music, M.D., DTPH (Lond.)

B. Home Address: 9 Deaver Place, Wyncote, PA 19095-1726

C. Home Telephone: 215-376-0223

II. EDUCATION

Institution	Date	Major/Minor Courses	Degree
University of London; London School of Hygiene and Tropical Medicine	1975-1976	Tropical Public Health	Diploma
Centers for Disease Control and Prevention – Atlanta, GA	1972-1973	Preventive Medicine	Resident
University of Maryland School of Medicine; Baltimore, MD	1966-1970	Fellow in Infectious Disease; Assistant Resident in Internal Medicine; Junior Assistant Res in Internal Medicine; Intern in Internal Medicine	Fellow, Resident, Intern
University of Maryland School of Medicine; Baltimore, MD	1962-1966	Doctor of Medicine	MD
George Washington University Washington, DC	1962	Invertebrate zoology and entomology	BS
George Washington University Washington, DC	1961	Liberal arts	AA

III. MERCK/MRL EMPLOYMENT HISTORY

Title	From – To
Director, Report Evaluation and Safety Surveillance Worldwide Product Safety and Epidemiology Department Merck Research Laboratories, Blue Bell, PA	May 1999 to present

IV. NON-MERCK EMPLOYMENT HISTORY

Title	From - To
Medical Epidemiologist Division of Women's and Children's Health, Department of Health and Human Services; State of North Carolina; Raleigh, NC	June 1998 to May 1999
Chief, Occupational and Environmental Epidemiology Division of Epidemiology, Department of Health and Human Services; State of North Carolina; Raleigh, NC	November 1996 – May 1998
Senior Regional Advisor for the Caucasus and Embassy Physician; United States Agency for International Development and American Embassy; Tbilisi, Republic of Georgia	1995 - 1996
Administrator, Division of Preventive Medicine and State Epidemiologist; then State Health Officer (last 6 months) Department of Health, State of Wyoming; Cheyenne, WY	1991 - 1994

Director, Global EIS Program; CDC, USPHS; Atlanta, GA	1986 - 1990
Deputy Director, Global EIS Program; CDC, USPHS; Atlanta, GA	1983 - 1986
Staff Epidemiologist, Policy Unit Population, Health and Nutrition Department, World Bank; Washington, DC	1982 – 1983
Deputy Director; Field Services Division, Atlanta, GA	1977-1982
Assistant Director; Field Services Division, Atlanta, GA	1976-1977
Full Time Internal Student, CDC Career Development; University of London, School of Hygiene and Tropical Medicine	1975 – 1976
Smallpox Eradication Advisor; Dacca, Bangladesh	1973 – 1975
Epidemic Intelligence Service Officer; Florida Department of Health and Rehabilitative Services; Jacksonville, FL	1971 – 1973

V. ACADEMIC EXPERIENCE

Instructor, Division of Infectious Diseases; University of Maryland School of Medicine; Baltimore, MD	1970 – 1971

VI. ADDITIONAL TRAINING

Source	Date	Type	Certification
American Management Association	1980	3 week course	Yes
Oak Ridge Nuclear Facility: Response to Nuclear Disaster	1992	1 week course	Yes

VII. SOCIETY MEMBERSHIPS and OTHER PFOFESSIONAL EXPERIENCES

Member, Armed Forces Epidemiology Board, US Department of Defense; 1998 to present
Georgian Academy of Sciences of Preventive Medicine and Human Ecology; 1996
Fellow, American College of Preventive Medicine; 1979
Diplomate, American Board of Preventive Medicine; 1978
Fellow, Royal Society of Hygiene and Tropical Medicine; 1975
Chairman, Scientific Advisory Committee; 1989-1991
Member, Scientific Advisory Committee, Caribbean Epidemiology Center (PAHO), Port of Spain, Trinidad and Tobago; 1988-1991
Consultant, Assessment of Health Needs, USAID Assessment Team, Sultanate of Oman, 1980
WHO Epidemiological Services Consultancies: Indonesia, 1979; Indonesia, Burma, Bangladesh, 1978; Republic of Korea, 1977
Post-liberation Nutrition Survey, CDC Assessment Team, Bangladesh; 1972
Research Physician, Infectious Diseases Hospital, University of Chile, Santiago, Chile; 1970
Attending Physician, Cholera Hospital, Pakistan-SEATO Cholera Research Laboratory; Dacca, East Pakistan; 1967-1968

VIII. HONORS

US Public Health Service Meritorious Service Medal - 1997
US Public Health Service Outstanding Service Medal - 1985
US Public Health Service Commendation Medal - 1979

IX. PUBLICATIONS

1. Music, S. I., Wenzel, R. P., Libonati, J. P., Snyder, M. J., Hornick, R. B., Woodward, T. E; Induced Human Cholera (abstract), Journal of Clinical Investigation, 49:69-70a, June 1970
2. Hornick, R. B., DuPont, H. L., Music, S. I., Snyder, M. J., Libonati, J. P.; Investigations into the Pathogenesis of Diarrheal Diseases, Trans Am Clin Climatol Assoc, Oct 26;82:141-7 1970.
3. Clyde, D. F., Miller, R. M., Music, S. I., McCarthy, V. C.; Prophylactic and Sporontocidal Treatment of Chloroquine-Resistant Plasmodium Falciparum from Viet Nam, American Journal of Tropical Medicine and Hygiene, 20:1-5, January, 1971.
4. Music, S. I., Fine, E.M., Togo, Y; Zoster-Like Disease in the Newborn due to Herpes Simplex virus, New England Journal of Medicine, 284:24-26, January 7, 1971.
5. Hornick, R. B., Dupont, H. L., Music, S. I., Snyder, M. J., Libonati, J. P.; Investigations into the Pathogenesis of Diarrheal Diseases, Trans AM Clin Climatol Assoc, 82: 26 October 1970.
6. Hornick, R. B., Music, S. I., Wenzel, R. P., Cash, R., Libonati, J. P., Snyder, M. J.; Woodward, TE; The Broad Street Pump Revisited: Response of Volunteers to Ingested Cholera Vibrios, Bull NY Acad Med, 47 (10): October, 1971.
7. Termini, B. A., Music, S. I.; The Natural History of Syphilis: A Review, South Med J., 65 (2): February, 1972.
8. Snyder, M. J., et al; Trimethoprim-sulfamethoxazole in the Treatment of Typhoid and Paratyphoid Fevers, J Infect Dis 128:Suppl:734-7: November, 1973.
9. Music, S. I., Howell, J. T., Brumback, C. L.; Red Tide, Its Public Health Implications, JFMA 60(11): November, 1973.
10. Cash, R. A., Music, S. I., Libonati, J. P., Snyder, M. J., Wenzel, R. P., Hornick, R. B.; Response of Man to Infection with *Vibrio cholerae*. I. Clinical, Serologic, and Bacteriologic Responses to a Known Inoculum, J. Infect Dis 129 (1): January, 1974.
11. Hattwick, M. A. W., Rubin, R. H., Music, S. I., Sikes, R. K., Smith, J. S., Gregg, M. B.; Postexposure Rabies Prophylaxis with Human Rabies Immune Globin, JAMA, Vol 227: 407-410: Jan. 28, 1974.
12. Cash, R. A., Music, S. I., Libonati, J. P., Craig, J. P., Pierce, N. F., Hornick, R. B.; Response of Man to Infection with *Vibrio cholerae*. II. Protection from Illness Afforded by Previous Disease and Vaccine, J. Infect Dis 130 (4): October, 1974.
13. Cash, R. A., Music, S. I., Libonati, J. P., Schwartz, A. R., Hornick, R. B.; Live Oral Cholera Vaccine: Evaluation of the Clinical Effectiveness of Two Strains in Humans, Infect Immun 19(4): Oct., 1974
14. Snyder, M. J., Gonzalez, O., Palomino, C., Music, S. I., et al: Comparative Efficacy of Chloramphenicol, Ampicillin, and Co-Trimoxazole in the treatment of Typhoid Fever. The Lancet, 2 (7966): 1155-7, Nov 27, 1976.
15. Music, S. I.: Surveillance, chapter in Guidelines for Analysis of Communicable Disease Control Planning in Developing Countries, International Health Planning Methods Series, Office of International Planning Methods Series, Office of International Health, USPHS, 1979 DHEW Publication No. (PHS) 79-50080.
16. Thacker, S. B., Music, S. I., Pollard, R. A., Berggren, G., Boulos, C., Nagy, T., Brutus, M., Pamphile, M., Ferdinand, R. O., Joseph, V. R.; Acute Water Shortage and Health Problems in Haiti, Lancet, 1:471-473, March 1, 1980.
17. Music, S. I.: The Role of Epidemiology in Helping CDC Improve Public Health. Annales Istituto Superiore di Sanitá, 21(4): 431-4, 1985.
18. Schwartz, B., Al-Tobaiqi, A., Al-Ruwais, A., Fontaine, R. E., A'ashi, J., Hightower, A. W., Broome, C. V., Music, S. I.; Comparative Efficacy of Cephtriaxone and Rifampicin in Eradicating Pharyngeal Carriage of Group A *Neisseria meningitidis*, Lancet, 1:1239-42, June 4, 1988.
19. Music, S. I.: Schultz, M. G.: Field Epidemiology Training Programs, New International Health Resources, JAMA, Vol 263, No 24:3309-3311, June 27, 1990.
20. Simonsen, L., Khan, A. S., Gary, H. E. Jr., Hanson, C., Pallansch, M. A., Music, S., Holman, R. C., Stewart, J. A., Erdman, D. D. Arden, N. H., Arenberg, I. K., Schonberger, L. B.; Outbreak of Vertigo in Wyoming: Possible Role of an Enterovirus Infection. Epidemiol Infect 117(1):149-57, August, 1996.
21. Music, S. I., Khetsuriani, N.: Epidemiology Bulletin, Ministry of Health, Republic of Georgia. Vol 1, Nos. 1-6:1-120, January – June 1996. (Though listed officially as *CDC Advisor* my actual role was to first do and then train others in how to do every step from conception and writing through publication and distribution of the first six monthly issues of this official publication of the Georgian government. These are available in English via Internet and the CDC homepage on SANet: http://www.sanet.ge/cdc/index.html.

22. Music, S. I., Georgia's public-health problems. Ltr to the Editor in Lancet, 348 (9043), Dec 21, 1996.
23. Smith, C. G., Music, S. I.: Pfiesteria in North Carolina: The Medical Inquiry Continues. NC Medical Journal, 59(4), Jul-Aug 1998.
24. Furney, W., Music, S. I., Wiley, J.: North Carolina Childhood Asthma Management Initiative: A Summary of the Summary Report. NC Medical Journal, 60(4), Jul-Aug 1999.
25. Music, S.I.: The Elimination of Preventable Asthma: Lessons from Smallpox. NC Medical Journal, 60(4), Jul-Aug 1999.
26. Khetsuriani, N., Music, S., Deforest, A., Sutter, R.W.: Evaluation of a Single Dose of Diphtheria Toxoid Among Adults in the Republic of Georgia, 1995: Immunogenicity and Adverse Reactions. Journal of Infectious Diseases 181 (Suppl 1):S208-S212, February 2000.

CURRICULUM VITAE

NAME:	BARRETT-CONNOR, Elizabeth Louise
WORK ADDRESS:	University of California, San Diego School of Medicine Department of Family and Preventive Medicine, 0607 La Jolla, California 92093-0607
HOME ADDRESS:	6423 Avenida Cresta La Jolla, California 92037-6514
BIRTHPLACE: & DATE:	Evanston, Illinois April 8, 1935
MARITAL STATUS:	Married, James D. Connor, M.D. 3 children
COLLEGE:	Mount Holyoke College, South Hadley, Massachusetts, 1952-1956 - Zoology
MEDICAL SCHOOL:	Cornell University Medical College, New York City, 1956-1960 - Medicine
INTERNSHIP:	University of Texas, Southwestern Medical School, Dallas, Parkland Memorial Hospital, 1960-1961
RESIDENCY:	University of Texas, Southwestern Medical School, Dallas, Parkland Memorial Hospital, 1961-1963
	University of Miami, School of Medicine, Jackson Memorial Hospital, Infectious Diseases, 1963-1964
POST-DOCTORAL:	London School of Hygiene & Tropical Medicine 1964-1965 – D.C.M.T., Diploma in Clinical Medicine of the Tropics
	University of Minnesota, Minneapolis, 1967 (3-week course) Advanced Epidemiology - Certificate
	Johns Hopkins University, Bar Harbor, Maine, 1968 (2-week course) Genetics - Certificate

FELLOWSHIPS:

Medical Student Fellowship in Public Health and Preventive Medicine, Cornell University Medical College, 1958
Louisiana State University Interamerican Program in Central America, Summer 1962
National Institutes of Health Post-Doctoral Fellowship, London School of Hygiene and Tropical Medicine, 1964-5
Fulbright Award (declined), 1964

DEGREES:

B.A., Mount Holyoke College, 1956
M.D., Cornell University, 1960
D.C.M.T., London School of Hygiene and Tropical Medicine, 1965

FACULTY APPOINTMENTS:

University of Miami, School of Medicine
Instructor of Medicine, 1965-1968
Assistant Professor of Medicine, 1968-1970

University of California, San Diego School of Medicine
Assistant Professor of Community Medicine and Medicine, 1970-1974
Associate Professor of Community and Family Medicine and Medicine, 1974-1981
Chief, Division of Epidemiology 1974-present
Professor of Family and Preventive Medicine and Medicine, 1981-present
Acting Chair, Department of Community and Family Medicine, 1981-1982
Chair, Department of Family and Preventive Medicine, 1982-1997

HONORS, NAMED LECTURESHIPS, AND VISITING PROFESSORSHIPS:

Frederick Murgatroyd Prize, London, 1965
Invited Participant, Bicentennial Colloquium of the New York Hospital, 1971
Invited Participant, Meeting Commemorating the 25th Anniversary of Dr. Donald W. Seldin's Chairmanship of the Department of Internal Medicine, The University of Texas, Dallas, 1977
Invited Participant, Symposium on the Advances in Diabetes Epidemiology, Colloquium Inserm, NIH, OMS. Abbaye de Fontevraud, France, May 3-7, 1982
Kaiser Award for Excellence in Teaching, University of California San Diego, School of Medicine, 1982

Living Legacy Award, Women's International Center, San Diego, California, March 6, 1984
Alexander D. Langmuir Lecture, Centers for Disease Control, Atlanta, April , 1985
Honorary Doctor of Science Degree, Mount Holyoke College, South Hadley, Massachusetts, May 26, 1985
Doctor of the Year Award, San Diego Health Care Association, San Diego, November 18, 1985
Katharine Boucot Sturgis Lecture, American College of Preventive Medicine, Atlanta, April 5, 1986
Kelly West Memorial Lecture/Award, American Diabetes Association, Indianapolis, June 6, 1987
Merit Award, National Institute of Aging, July, 1987 –1996
Visiting Professor, Royal Society of Medicine, London, May 1989.
John Rankin Lecture, Madison, Wisconsin, October 20, 1989
Don McLeod Memorial Lecture, Halifax Nova Scotia, February 9, 1990.
Member, Institute of Medicine, 1991
Elizabeth Blackwell Lecture, Rochester, Minnesota, September 18, 1991
The Lila Wallace Visiting Professorship, The New York Hospital/Cornell Medical Center, March 4-5, 1992
The Donald P. Shiley Visiting Lectureship, Scripps Clinic and Research Foundation, San Diego, March 13, 1992
Outstanding Educator Award, Association of Teachers of Preventive Medicine, March 22, 1992
Leonard M. Schuman Lecture, University of Michigan, Ann Arbor, July 28, 1993
Wade Hampton Frost Lecture, American Public Health Association, San Francisco, October 25, 1993
Joe Stokes Lecture Research Seminar, Grand Rounds, Boston, November 11, 1993
University of California, San Diego, Faculty Research Lecturer Award, March 10, 1994
James D. Bruce Memorial Award, American College of Physicians, April 21, 1994
Soroptimist International of La Jolla Award, Making a Difference for Women, Health, June 7, 1995
Ancel Keys Lectureship, American Heart Association Scientific Sessions, November 13, 1995
American Heart Association, Elizabeth Barrett-Connor Research Award in Epidemiology and Prevention for Investigators In Training, November 14, 1995
UCSD Chancellor's Associates Faculty Excellence Award in Research, January 31, 1996
Honorary Doctor of Medicine Degree, University of Utrecht, The Netherlands, March 26, 1996
Honorary Doctor of Medicine Degree, University of Bergen (Norway), August 3, 1996
The Florence Mahoney Lecture on Aging, National Institures of Health, September 25, 1996
Arthur Gordon Visiting Professor, University of California, Los Angeles, October, 1996
American Heart Association Council on Epidemiology and Prevention, Distinguished Service Award, November 12, 1996

The Donald P. Shiley Visiting Lectureship, Scripps Clinic and Research Foundation, March, 1997
The Cleveland Clinic Foundation Department of Cardiology Visiting Professor, June, 1997
John Cassell Memorial Lecture, Society for Epidemiologic Research, 30th Annual Meeting, June 12, 1997
Clinical Service Award, Society for the Advancement of Women's Health Research, June 24, 1997.
Raine Distinguished Visitor's Award, The University of Western Australia, October 26 - November 5, 1997
Distinguished Lecturer in Geriatrics, Duke University Medical Center, J January 29-30, 1998
13th Annual Harry S. Feldman Lecture, American Epidemiologic Society (AES) Meeting, Harvard Medical School, March 26, 1998
Award of Meritorious Achievement of the American Heart Association, Dallas, Texas, June 26, 1998
Women's Health Hero Award – American Health for Women, New York, September, 1998
Woman in Science Award – American Medical Women's Association, New Orleans, November, 1998
Nathan J. Kiven Oration and Brownwide Grand Rounds, The Miriam Hospital and Brown University,Rhode Island, April 9, 1998
Alvin L. Schultz Visiting Professor of Internal Medicine, Minneapolis, October 20, 1998
Visiting Professor, Brigham and Women's Hospital, Boston, Massachusetts, November, 1998
National Institutes of Health Award for Outstanding Work in Gender Differences in Osteoporosis, March, 1999
Heath Clark Lectureship, London School of Hygiene and Tropical Medicine, London, England, March, 1999
Invited Participant, Controversies and Dilemmas in Endocrinology, Royal College of Physicians of Edinburgh, Scotland, March, 1999

GRANTS:

National Institutes of Health, Lipid Research Clinic,
Veterans Administration Hospital, La Jolla, California, 1970-1989 .
Janssen Drug Study Fund, 1976-1978.
National Institutes of Health, Peripheral Arterial Disease
Grant #HL22255-0l, April l, 1978, - November 30, 1980.
American Heart Association, California Affiliate,
Grant-in-Aid, #80-S114, July l, 1980 - June 30, 198l.
National Institute of Arthritis, Diabetes, Digestive &
Kidney Diseases, Epidemiology of Diabetes in an Adult
Community #1 RO1 AM31801, July 1, 1983 - June 30, 1988.

UCSD/SDSU Teaching Nursing Home Project. #NIA AG03990-01A1, May 1, 1984 - April 30, 1989.

National Institutes of Health, National Heart, Lung and Blood Institute, PHS HL34591, Endogenous Sex Hormones & Cardiovascular Disease Risk in Men, April 1, 1986 - March 31, 1987.

American Heart Association California Affiliate, Orange County Chapter Grant-in-aid-Dietary Factors, Blood Pressure and Cardiovascular Disease. #85-S116, July 1, 1986 - December 31, 1988.

Weight Watchers - Analyzing in Detail Extensive Database with Regard to Obesity and Heart Disease, January 1,1987 - December 31, 1989.

National Institute of Health - National Institute of Aging- Study of Risk Factors for Osteoporosis in the Elderly. #NIH/NIA 1 R37 AG07181-01, UCSD #90-6518, August 1, 1987 - July 31, 1992 (Merit Award).

National Institute of Health - Postmenopausal Estrogen/ Progestin Interventions (PEPI). #NIH 1001- HL40207-01, UCSD #90-6500. September 3, 1987 - August 31, 1992.

University of California Academic Geriatric Resource Program-Interdisciplinary Geriatrics Fellowship Program. #87SD-C2D-2-01, July 1, 1987 - June 30, 1988.

National Institute of Diabetes and Digestive and Kidney Diseases - Epidemiology of NIDDM and IGT in an Adult Community. UCSD #88-5256, July 15, 1988 - June 30, 1990.

American Association of Retired Persons.- The Effects of Husbands' Retirement on Their Wives. UCSD #87-6259, January 1, 1988 - December 31, 1988.

National Institute of Health, NHLBI - LRC Follow-up Study--CPPT and Prevalence. UCSD #6947, June 29, 1971 - September 30, 1991.

National Institute of Health, NIA - Alzheimers Disease Research Center Competitive Supplement. UCSD #89-6638, August 17, 1990 - March 31, 1994.

National Institute of Health - Predictors of Cardiovascular Disease in the Elderly. UCSD #90-6070, January 1, 1991 - December 31, 1991.

National Institute of Health, NIDDK - Epidemiology of NIDDM and IGT in an Adult Community. UCSD #91-6083, December 1, 1991 - November 30, 1996.

National Institute of Health - Epidemiology of NIDDM and IGT Supplement. UCSD #92-6591, June 1, 1992 - February 28, 1993.

National Institute of Health, NIA - Study of Risk Factors for Osteoporosis

in the Elderly (Osteo II). UCSD #91-6122. August 1, 1992 - July 31, 1997. (Merit Award)

Merck, Sharp and Dohme, Fracture Intervention Trial (FIT). UCSD #92-5548, October 1, 1991 - March 31, 1997.

National Coffee Association, "Coffee/Caffeine/Bone Mineral Density". UCSD #92-6164, February 1, 1992 - January 31, 1993 (no cost extension August 31, 1993).

Solvay Pharmaceuticals - A Double-Blind, Parallel Group Study of the Effects of Estratest H.S. vs. Premarin in Surgically Menopausal Women. UCSD #92-6838. June 1, 1992 - May 31, 1995.

Wyeth-Ayerst, Heart & Estrogen/Progestin Replacement Study (HERS). UCSD #91-5180. October 8, 1992 - December 31, 1998.

National Institute of Health, NHLBI - Postmenopausal Estrogen/Progestin Interventions (PEPI). UCSD #92-5242. August 1, 1992 – July 31, 1994.

Weight Watchers. Sex hormones, obesity and diabetes in older women. UCSD #93-7168. November 1, 1993 - October 31, 1994.

National Institutes of Health, NIDDK. NIDDM Primary Prevention Trial. UCSD #94-5368, July 1, 1994 to June 30, 2001. (Co-PI)

National Institute of Health, NHLBI, Postmenopausal Estrogen-Progestin Intervention (PEPI) Safety Followup Study, N01-HV-48136, June 15, 1994 to December 14, 1997.

National Institute of Health, NHLBI, Postmenopausal Estrogen-Progestin Intervention (PEPI) Safety Followup Analysis Study, N01-HV-48136, August 1, 1994 to July 31, 1997.

Lilly Research Laboratory. Comparison of Raloxifene HCL and Placebo in the Treatment of Postmenopausal Women with Osteoporosis. UCSD #95-5368, November 1, 1994 to October 31, 1999.

Wyeth-Ayerst Laboratories. A Randomized, Double-Blind Placebo & Active Controlled, Parallel, Multicenter Study to Assess the Safety & Efficacy of 3 1/2 Day Combinations of 17B-Estradiol Norethindrone Acetate Transderma Delivery Systems for Relief of Menopausal Vasomotor Symptoms & Reduction of Endometrial Hyperplasia. UCSD#97-9150. May 27, 1997 to April 30, 1999.

Osteometer Meditech A/S. Bone Mineral Content & Density in the Forearm, Speed of Sound, & Boradband Ultrasound Attenuation in the Calcancus: Normal Range in US Caucasian Females & Males, 20-80 years of age. UCSD #98-9010. June 15, 1997 to December 31, 1997.

Osteometer Meditech A/S. Forearm Mineral Density in the Normal Caucasian Female Population in the Calcancus: Normal Range in US Caucasian Females & Males, 20-80 Years of Age. UCSD 97-9099. December 15, 1997 to January 31, 1997.

Merck & Co. A 5-Year, Double-Blind, Randomized, Placebo-Controlled Extension Study to Examine the Long-Term Safety & Efficacy of Oral Alendronate In Postmenopausal Women Who Previously Received Alendronate in Conjunction with the Fracture Intervention Trial (FLEX) UCSD #98-9051. January 3, 1998 to October 30, 2003.

National Institutes of Health, Soy Health Effects (SHE). 1RO1 HL57790-01,.April 1, 1997 to March 31, 2000.

National Institutes of Health/NIDDK, Diabetes Primary Prevention Program (DPP). 5UO1 DK48339-04, September 10, 1994 to June 30, 2001.

National Institutes of Health, Comparison of Medical and Surgical Treatment for Abnormal Uterine Bleeding Post-Menopausal Women (Ms?). September 30, 1996 to September 29, 2001.

Eli Lilly & Co. Raloxifene Hydrochloride or Placebo in Postmenopausal Women At Risk for Major Cardiovascular Events. UCSD #98-9146. September 4, 1998 – September 30, 2005.

National Institutes of Health, NIA. Gender Differences in Osteoporosis (OSTEO III) UCSD #98-6285. December 1, 1998 to November 30, 2002.

National Institutes of Health, Osteoporotic Fractures in Men (MR.OS). UCSD #98-6088. December 10, 1998 to November 30, 2003.

MEDICAL QUALIFICATIONS:

Licensure, Florida, 1965
Licensure, California, 1970 (#C-32076)
Diplomate, American Board of Internal Medicine, 1968
Diplomate, National Board of Medical Examiners

PROFESSIONAL SOCIETY MEMBERSHIPS:

Fellow, American College of Physicians (Publications Committee, 1988-90)
Fellow, Council on Cardiovascular Epidemiology, America Heart Association (Chair, 1989)
Fellow, Royal Society of Health
Fellow, American College of Preventive Medicine
Fellow, American College of Nutrition
Fellow, The Royal Society of Medicine
Member, American Venereal Disease Association (Vice-President, 1977-1978)
Member, American Federation for Clinical Research
Member, Association of Teachers of Preventive Medicine (Board of Directors, 1987-90)
Member, Infectious Disease Society of America

Member, International Epidemiological Association
Emeritus Member, American Society of Tropical Medicine and Hygiene
Member, Society for Epidemiologic Research (President, 1983)
Member, Association for Practitioners in Infection Control
Member, California Academy of Preventive Medicine
Member, Western Association of Physicians
Member, American Epidemiological Society (President, 1993-94)
Member, American Diabetes Association
Consultant, Veterans Administration Hospital, Miami, 1969
Consultant/Lecturer in Internal Medicine (Infectious Diseases), U.S. Naval Hospital, San Diego, 1970-85
Consultant, Mercy Hospital, San Diego, 1970-85
Consultant, American Medical Association Department of Drugs, Chicago, 1976
Member, Hospital Infection Control Committee, University Hospital, San Diego, 1970-1972 (Chairman 1975-1977)
Member, Hospital Infection Control Committee, Veterans Administration Hospital, La Jolla, 1971-85
Member, Research Committee, Zoological Society of San Diego, 1978-86
Member-at-Large, Research Peer Review Sub-Committee, American Heart Association, California Affiliate, 1977-1981
Member, Advisory Committee for Genetic Disorders, California Department of Health, 1974-1975
Ad hoc member, Study Section, Center for Disease Control, Atlanta, 1971-1972
Member, Expert Advisory Committee, Food & Drug Administration, Rockville, 1972-1977
Member, Advisory Council on Immunization Practices, Center for Disease Control, Atlanta, 1973-1977
Member, Preventive Medicine and Public Health Test Committee, National Board of Medical Examiners, Philadelphia, 1974-1980 (Chair, 1977-1980)
Member, Epidemiology Working Group, National Commission on Arthritis and Musculoskeletal Diseases, Boston, 1975-1976
Ad hoc Member, National Institute of Allergies and Infectious Diseases Committee, HEW/NIH, 1977
Member, Consultant Task Force for the Study of Health in Egypt and Future U.S. Development Assistance
Alternatives, National Institute of Medicine, 1978
Member, National Institute of Allergies and Infectious Diseases

Committee, 1978-1982
Member, American Tropical Medicine Delegation to China, American Society of Tropical Medicine and Hygiene, 1978
Member, The American Geriatrics Society, 1987-present
Member, Medical Research and Development Advisory Panel, Review Group Concerned with Parasitic Diseases, Walter Reed Army Institute of Research, Department of the Army, 1979-1982
Member, Special Consultants to Department of Defense Overseas Medical Research Laboratories, US Department of Defense, 1980
Consultant, Task Force, Institute of Medicine, Division of International Health, Health in Egypt: Recommendations for U.S. Assistance, January, 1979
Member, Core Faculty, Annual Seminars on Epidemiology of Cardiovascular Disease, American Heart Association, 1978-present
Member, California Medical Association Scientific Advisory Panel; Preventive Medicine and Public Health, 1982-present
Member, American Epidemiological Society Membership Committee 1987-present
Member, Advisory Committee, Role of BCG Vaccinations in the United States, Research Foundation, 1983-1985
Member, (San Diego) Mayor's Task Force for Acquired Immunity Deficiency Syndrome (AIDS), 1983-1985
Member, Epidemiology Research Unit, University of Texas, 1983-1986
Member, National Advisory Committee on Vital and Health Statistics, May 30, 1984 - February 28, 1987
Member, American Public Health Association, Epidemiology Section, (Chair, 1989-91)
Member, European Diabetes Epidemiology Study Group, 1984- present
Member, NHANES III Advisory Committee (FACEB), 1985
Member, Preventive Medicine Residency Advisory Committee, San Diego (Chair, 1985)
Member, Epidemiology and Biometry Program Working Group, Subcommittee of the Clinical Applications and Prevention Advisory Committee (CAPAC), National Heart, Lung, and Blood Institute, Bethesda, Maryland, 1985-1987
Member, Burroughs-Wellcome Fund/American College of Preventive Medicine Pharmacoepidemiology Award Advisory Committee, 1986-1989
Member, San Diego Foundation for Medical Care, 1986-present
Member, Resource Advisory Committee on the Epidemiology of the

Chronic Diseases of Aging of the National Archives of Computerized Data on Aging, 1988-1994
Member, Technical Advisory Committee for Diabetes Translation and Community Control Programs, Centers for Disease Control, February 6, 1989 - June 30, 1991.
Member, International Epidemiological Association (North American Councillor, 1990-present)
Member, International Scientific Committee for the 3rd International Conference on Preventive Cardiology, 1989-1990
Member, U.S. Army Research and Development Advisory Committee, Ft. Detrick, Frederick, Maryland 1990-1993
Member, International Society and Federation of Cardiology, Section of Epidemiology, 1990-present
Member, National Heart, Lung, and Blood Institute Task Force on Hypertension 1990-93
Member, National Diabetes Advisory Board, National Institutes of Health, 1990-1994
Member, The Royal Society of Medicine, 1992-present
Member, Advisory Board of the HERITAGE Study, 1992-present
Member, Faculty, WHO Postgraduate Seminar on Diabetic Epidemiology (Krakow, Poland), 1992
Member, Data and Safety Monitoring Board, Women's Health Initiative, 1993-present
Member, Faculty of International Society & Federation of Cardiology Teaching Seminar 1993-present
Councilor, Western Association of Physicians, 1994-97
Member, Human Subjects Program Review Committee, UCSD, 1994-present
Member, The New York Academy of Sciences, 1995-present
Member, Scientific Advisory Board, Ostex International, Inc., 1995-present
Member, Raloxifene Advisory Board, Eli Lilly and Company, 1995-present
Member, American Federation for Aging Research, National Scientific Advisory Committee, 1996
Member, Membership Committee, Institute of Medicine, 1996-1999
Member, Armed Forces Epidemiology Board, 1996 -
Member, Advisory Council, National Instiurte of Aging-1996-
Member, Advisory Council, National Institute of Aging, 1997 –
Board of Directors, North American Menopause Society, 1997-
National Institues of Health/Women's Health Initiative: Data and Safety Monitoring Board, 1997-

Member, Editorial Board, American Journal of Preventive Medicine, 1998-
Sigma Xi – The Scientific Research Society, 1998 –
Member, National Lipid Education Council, 1998-
Member, Science Advisory Board, County of San Diego, 1999-
Member, Medical Committee, Royal Netherlands Academy of Arts and Sciences, 1999-
Member, Endocrine Society, 1999-

REVIEWER:

Annals of Internal Medicine, 1974-present
Review of Respiratory Diseases, 1974-present
New England Journal of Medicine, 1974-present
Journal of American Medical Association, 1975-present
Public Health Reports, 1975-1985
Emergency Medicine, 1975-1980
Western Journal of Medicine, 1975-present
American Journal of Tropical Medicine and Hygiene, 1979-present
Arthritis and Rheumatism, 1981-present
American Journal of Epidemiology, 1981-present
Reviews of Infectious Diseases, 1982-present
Arteriosclerosis, 1984-present
Circulation, 1985-present
Journal of Chronic Disease, 1982-present
Preventive Medicine, 1988-present
International Journal of Gynecology & Obstetrics, 1994-present

EDITORIAL BOARDS:

American Journal of Epidemiology
American Journal of Infection Control, 1981-1986
American Journal of Preventive Medicine
Annals of Epidemiology
Annals of Internal Medicine, 1979-82
Cardiovascular Risk Factors, 1995-present (Member of Advisory Board)
Circulation
International Journal of Epidemiology
Journal of Clinical Investigation (Consulting Editor), 1995-1997
Reviews in Clinical Gerontology
Sexually Transmitted Diseases, 1977-81
The Women's Letter
Menopause

November 1999

CURRICULUM VITAE

Name: Philip J. Landrigan, M.D., M.Sc., D.I.H.
SSN: 022-32-0504

Born: Boston, Massachusetts, June 14, 1942

Wife: Mary Florence

Children: Mary Frances
Christopher Paul
Elizabeth Marie

Education:

High School: Boston Latin School, 1959
College: Boston College, A.B. (magna cum laude), 1963
Medical School: Harvard - M.D., 1967
Internship: Cleveland Metropolitan General Hospital, 1967-1968
Residency: Children's Hospital Medical Center, Boston, (Pediatrics), 1968-1970
Post Graduate: London School of Hygiene & Tropical Medicine, 1976-77
Diploma of Industrial Health (England), 1977
Master of Science in Occupational Medicine, University of London (with distinction), 1977

Positions Held:

Current:
Mount Sinai School of Medicine, Ethel H. Wise Professor of Community and Preventive Medicine and Chairman of the Department of Community and Preventive Medicine, 1990-Present.
Mount Sinai School of Medicine, Director, Division of Environmental and Occupational Medicine, Department of Community and Preventive Medicine, 1985-Present.
Mount Sinai School of Medicine, Professor of Pediatrics, 1985-Present.

Previous:
U.S. Environmental Protection Agency, Senior Advisor to the Administrator on Children's Health and the Environment, 1997-1998.
National Institute for Occupational Safety and Health, Director, Division of Surveillance, Hazard Evaluations and Field Studies, 1979-1985.
Centers for Disease Control, Chief, Environmental Hazards Activity, Cancer and Birth Defects Division, Bureau of Epidemiology, , 1974-1979.
Centers for Disease Control, Director, Research and Development, Bureau of Smallpox Eradication, 1973-1974.
Centers for Disease Control, Epidemic Intelligence Service (EIS) Officer, 1970-1973.

Adjunct Positions:

University of Washington School of Public Health and Community Medicine, Clinical Professor of Environmental Health, 1983 - Present.
Harvard Medical School, Visiting Lecturer on Preventive Medicine and Clinical Epidemiology, 1982 - Present.
Harvard School of Public Health, Visiting Lecturer on Occupational Health, 1981 - Present.
University of Cincinnati, Department of Environmental Health, College of Medicine, Assistant Clinical Professor of Environmental Health, 1981 - 1986.
London School of Hygiene and Tropical Medicine, Visiting Fellow, TUC Institute of Occupational Health, 1976 - 1977.
Harvard Medical School, Clinical Instructor in Pediatrics, 1969 - 1970.

Memberships:

American Academy of Pediatrics, Fellow
Society for Epidemiologic Research, Member
American Public Health Association, Member
Occupational Health Section, Chair, 1989-90
Royal Society of Medicine, Elected Fellow
International Commission on Occupational Health, Member
Scientific Committee on Epidemiology
American College of Epidemiology, Fellow
Board of Directors, 1990 - 1993.
American Epidemiological Society, Elected Member
Collegium Ramazzini, Fellow
President, 1997-present
Herman Biggs Society, Member
New York Academy of Sciences, Fellow
New York Occupational Medicine Association, Member
Board of Directors, 1988 - 1990.
American College of Occupational and Environmental Medicine, Fellow
New York Academy of Medicine, Elected Fellow
Physicians for Social Responsibility, Member
Board of Sponsors, 1994-95; Board of Directors 1996-1999

Specialty Certifications:

American Board of Pediatrics - 1973
American Board of Preventive Medicine:
General Preventive Medicine - 1979
Occupational Medicine - 1983

Awards and Honors:

Institute of Medicine, National Academy of Sciences, Elected to membership, 1987
U.S. Department of Health, Education and Welfare ,Volunteer Award, 1973
U.S. Public Health Service, Career Development Award, 1976
Centers for Disease Control, Group Citation as Member of Beryllium Review Panel, 1978
U.S. Public Health Service, Meritorious Service Medal, 1985
New York Committee for Occupational Safety and Health, Annual Honoree, 1985
New England College of Occupational and Environmental Medicine, Harriet Hardy Award, 1993
United Brotherhood of Carpenters, William Sidell Presidential Award, 1995
American Public Health Association, Herbert L. Needleman Medal and Award for Scientific Contributions and Advocacy on Behalf of Children, 1995.
International Association of Fire Fighters, Occupational Health and Safety Award, 1995
Physicians for Social Responsibility, Broad Street Pump Award in Environmental Health, 1996
Mayo Clinic, Department of Pediatrics, Amberg-Heimholtz Lecturer in Pediatrics, 1998
International Society for Occupational and Environmental Health, Vernon Houk Award, 1998
Centers for Disease Control and Prevention, Langmuir Memorial Lecturer, 1999
American College of Preventive Medicine, Katherine Boucot Sturgis Award, 1999
Mothers & Others for a Livable Planet, Award for Advocacy on Behalf of the Health of Children, 1999
Earth Day New York, Award for Excellence in Environmental Medicine, 1999

Visiting Professorships:

University of Tokyo, Visiting Professor of the Faculty of Medicine, September 1989
University of Tokyo, Visiting Professor of the University, July 1990
University of Cape Town Medical School, Visiting Professor, Department of Community Health, March 1992
Medical College of Pennsylvania, Catherine Boucot Sturgis Visiting Professor in Community and Preventive Medicine, March 1992
National University of Singapore, Visiting External Examiner in Occupational Medicine, 1994
Duke University Medical School, Visiting Professor, NIEHS Clinical Training Program in Environmental Medicine, 1995

Committees:

The White House
Presidential Advisory Committee on Gulf War Veterans' Illnesses, 1995-1996.

American Academy of Pediatrics
Committee on Environmental Hazards, 1976 - Present. Chairman, 1983-1987.

National Research Council

National Academy of Sciences, Assembly of Life Sciences. Board on Toxicology and Environmental Health Hazards, 1978-1987; Vice-Chairman, 1981-1984.

National Academy of Sciences, Assembly of Life Sciences, 1981-1982; Commission on Life Sciences, 1982-1984.

Institute of Medicine, Committee for a Planning Study for an Ongoing Study of Costs of Environment-Related Health Effects, 1979-1980.

National Academy of Sciences, Panel on the Proposed Air Force Study of Herbicide Agent Orange, 1979-1980.

National Academy of Sciences, Committee on the Epidemiology of Air Pollutants, Vice-Chairman, 1984-1985.

National Academy of Sciences, Committee on Neurotoxicology in Risk Assessment, 1987-1989.

National Academy of Sciences, Committee on the Scientific Issues Surrounding the Regulation of Pesticides in the Diets of Infants and Children, Chairman, 1988-1992.

National Academy of Sciences, Board on Sustainable Development, 1995-1998.

National Institutes of Health/U.S. Public Health Service

National Institutes of Health, Study Section on Epidemiology and Disease Control, 1986-1990.

National Institute of Environmental Health Sciences, Third Task Force for Research Planning in the Environmental Health Sciences; Chairman, Subtask Force on Research Strategies for Prevention of and Intervention in Environmentally Produced Disease, 1983-1984.

National Institute for Occupational Safety and Health, Board of Scientific Counselors, 1995-1997.

State and Local Government

State of New York, Governor's Blue Ribbon Committee on the Love Canal, 1978-1979.

State of New Jersey, Meadowlands Cancer Advisory Board, Chair, 1987-1989.

State of New York, Asbestos Advisory Board, Chair, 1987 - Present..

State of New York, New York State Advisory Council on Lead Poisoning Prevention, Chairman, 1993 - Present.

City of New York, Mayor's Lead Paint Poisoning Advisory Committee, 1991-1993.

State of New York, Public Health Priorities Committee, 1996.

State of New York, Health Research Science Board, 1997 - Present.

Academic

Harvard School of Public Health, Occupational Health Program, Residency Review Committee, 1981-1983; Chairman, 1981.

New York Academy of Medicine, Working Group on Housing and Health, 1987-1989; Chairman, 1989.

Association of University Programs in Occupational Health and Safety, 1985 – Present; President, 1986-1988.

New York Lung Association, Research and Scientific Advisory Committee, 1986-1989. Board of Directors, 1987-1990.

Milbank Memorial Foundation, Technical Board, 1986-1988.

Mickey Leland National Urban Air Toxics Research Center, National Advisory Committee, 1994-1995.

Cornell University, Dean's Advisory Council in Veterinary Medicine, 1996-1997.

International Organizations

World Health Organization. Contributor to the WHO Publication: "Guidelines on Studies in Environmental Epidemiology" (Environmental Health Criteria, No. 27), 1984.
International Agency for Research on Cancer, Working Groups on Cancer Assessment, October 1981 and June 1986. (IARC Monographs No. 29 and No. 42).

Environmental Organizations

INFORM, Board of Directors, 1991 - Present.
Environmental Health Foundation, Board of Directors, 1993 - Present.
Colette Chuda Environmental Fund, Scientific Advisory Committee, 1994 - Present.
Children's Health Environment Coalition, Board of Directors, 1996 - Present.
Children's Environmental Health Network, Board of Directors, 1995 - Present.

Labor Unions

United Automobile Workers (UAW) - Chrysler Corporation, Joint Scientific Advisory Committee, Member, 1990 - Present.
United Brotherhood of Carpenters, National Health and Safety Fund, Medical Advisory Committee, 1990 - Present; Chairman, 1994 - Present.
International Association of Fire Fighters, John Redmond Foundation, Medical Advisory Committee, 1989 - Present.
International Brotherhood of Teamsters, National Health and Safety Advisory Committee, 1994 - Present.
George Meany Center for Labor Studies, Board of Trustees, 1994-1997.

Other Organizations

Health Insurance Plan (HIP) of Greater New York, Board of Directors, 1992-1994.
American Legion, Science Panel, Chairman, 1988 - Present.

Editorial Boards:

Editor-in-Chief: *American Journal of Industrial Medicine*, 1992 - Present; Consulting Editor, 1979-1992.
Editor-in-Chief: *Environmental Research*, 1987-1994.
Consulting Editor: *Archives of Environmental Health*, 1982 - Present.
Editorial Board: *Annual Review of Public Health*, 1984-1990.
Senior Editor: *Environmental Research*, 1985-1987.
Editorial Board: *American Journal of Public Health*, 1987 - Present.
Editorial Board: *New Solutions*: A Journal of Environmental and Occupational Health Policy, 1990 - Present.
Editorial Board: *The PSR Quarterly*: A Journal of Medicine and Global Survival, 1990-1994.
Editorial Board, *Journal of Public Health Management and Practice*, 1995-1996.

National Service:

United States Public Health Service, Commissioned Corps, 1970-1985. LCDR (04) to CAPT (06).
United States Naval Reserve, Medical Corps, 1996 - Present.
LCDR (0-4) 1996-98; CDR (0-5) 1 April, 1998 - Present.

ACCUSATIONS-- SQUALENE

1. What is squalene?

Squalene is a naturally occurring substance found in plants, animals, and humans. It is manufactured in every human body as part of the process of making cholesterol and hormones. Squalene is also found in a variety of foods, cosmetics, health supplements, and over-the-counter medications. (Links to commercial squalene sources)

Squalene has been used as an adjuvant (a substance used to improve the body's response to a vaccine) in some investigational vaccines manufactured in the U.S., including vaccines to protect against HIV disease. Squalene is approved by European health agencies for use in an influenza vaccine. Whatever the arguments for or against squalene as a vaccine adjuvant, the fact is that none of the vaccines that were administered to U.S. troops during the Gulf War contained squalene as a vaccine adjuvant. This includes the anthrax vaccine, which does not contain squalene and never has contained squalene. The FDA has licensed only aluminum salts (e.g., aluminum hydroxide, aluminum phosphate, aluminum potassium sulfate) as adjuvants.

The Department of Defense (DoD) has never exposed any military member or civilian to any squalene-containing investigational product without the person's informed consent, abiding by FDA regulations. The DoD has conducted five human clinical trials using investigational vaccines containing squalene (investigational vaccines for the prevention of malaria and HIV infection) in FDA-approved vaccine studies. Two of the malaria vaccine studies involving a total of 17 human volunteers were conducted before or during the Persian Gulf War. Although it is unlikely, some of these subjects may have been involved in the Gulf War. Nevertheless, these investigational vaccines were part of FDA-approved studies that followed FDA guidelines for the use of investigational vaccines, including the informed consent of the participants.

2. Did DoD have anthrax vaccine tested for the presence of squalene?

Yes, and the vaccine was found to contain no squalene. To determine whether squalene was present in the anthrax vaccine, the DOD recently contracted with an independent civilian laboratory, Stanford Research Institute (SRI) International of Menlo Park, California, to test for the presence of squalene in every lot of the anthrax vaccine released to DOD. SRI International tested 14 lots of anthrax vaccine and formally reported that no squalene was detected in any of the 14 lots. The test they used is sensitive enough to detect the squalene naturally present in the oil in a human fingerprint. The DOD will test all other lots of anthrax vaccine in the stockpile when the allegations arose. Graphic images of the test results are posted at http://www.anthrax.osd.mil/Site_Files/lot_documents/lot_documents_menu.htm.

3. Has DoD ever requested that MBPI change the formula for licensed anthrax vaccine or develop a new anthrax vaccine to include squalene?

No. DoD never requested MBPI to change the formula for the licensed vaccine or to develop a new anthrax vaccine with any adjuvant, including squalene.

4. What are the facts behind the accusations about squalene?

In their effort to explain the health problems of some Gulf War veterans, a few investigators have theorized, and the press has amplified their theories, that a vaccine adjuvant may have caused an autoimmune disease in veterans. A recent *Vanity Fair* article "The Pentagon's Toxic Secret" (May 1999) alleges that the DoD possibly used "an illicit and secret anthrax vaccine" on its own soldiers. The writer's interpretation and presentation of the facts regarding the Department's use of anthrax vaccine are speculative, inflammatory, and wrong. His allegations and the reported "clinical evidence" are not new. Since 1997, reports in the *Washington Times* and its magazine *Insight on the News* have made similar allegations regarding an experimental "anti-HIV vaccine."

The investigators cited in the Vanity Fair and Insight on the News articles (Pamela Asa, Ph.D., Memphis, TN and Robert Garry, Ph.D., Tulane University School of Medicine, New Orleans, LA) report that they have developed and patented a test for anti-squalene antibodies. Autoimmune Technologies, LLC, of New Orleans, has an exclusive license on the use of the test. With their test the investigators report that they have detected anti-squalene antibodies in the blood of ill Gulf War veterans. Their method was published in the February 2000 issue of the journal "Experimental and Molecular Pathology." Whether or not this test has any clinical meaning will be settled by medical experts over time. For now, it is sufficient to recognize the conclusions of the authors: "It is important to note that our laboratory-based investigations do not establish that squalene was added as adjuvant to any vaccine used in military or other personnel who served in the Persian Gulf War era."

5. What did the GAO say about squalene testing and what are DoD researchers doing?

The U.S. General Accounting Office (GAO) has released a report "Gulf War Illnesses: Questions about the Presence of Squalene Antibodies in Veterans Can be Resolved" (GAO/NSIAD-99-5). The Department of Defense disagreed with the GAO's opinion that "the first step is to determine the extent to which they [antibodies to squalene] are present in a larger group of sick Gulf War-era veterans."

To investigate the squalene hypothesis, a scientifically proven test for squalene antibodies is needed to assess whether Gulf War veterans have antibodies to squalene. In response to a DoD solicitation for research on illnesses among Gulf War veterans, a DoD investigator and nationally recognized expert on antibodies to cholesterol and other lipids submitted a research proposal to determine the feasibility of developing a test for antibodies to squalene. The funded research project to determine whether antibodies to squalene exist has five main objectives: 1) Development and validation of an enzyme-linked immunosorbant assay (ELISA) for antibodies against squalene. 2) Evaluation and potential development of other assays for antibodies to squalene. 3) Development of a positive control antibody to squalene. 4) Production of the positive control antibody to squalene for use in the assays. 5) Testing of normal human serum for antibodies to squalene by ELISA and other methods. This study should provide adequate scientific evidence to resolve the issue of whether squalene antibodies exist and can be detected in human serum. Only if this kind of preliminary evidence indicates that it is possible to create and measure anti-squalene antibodies can one contemplate the next step. The next step would be to determine whether the presence of anti-squalene antibodies differs between two groups. For example, one might want to compare (1) deployed vs. nondeployed veterans, (2) veterans with vs. without symptoms attributed to Gulf War illnesses, or (3) some other comparison. These steps will take a couple years to work through.

The proper first step is to show that the test measures what the test claims to measure. Further, the medical significance and the origin of antibodies to squalene, even if their existence is corroborated, remain unknown. Without such information, Gulf War veterans get only speculation about the meaning of the test result and its implication for their health. Gulf War veterans deserve objective evidence and recommendations based on sound science.

6. What does the U.S. Senate say about squalene?

In its investigations of illnesses among Gulf War veterans, the Senate Special Investigations Unit (SIU) found no credible information indicating that vaccines used during the Gulf War contained squalene (1998, page 123). In its report, the SIU stated that according to the Food and Drug Administration (FDA), squalene can be contained in a vaccine due to two different processes: 1) as an adjuvant, which is an agent to enhance the immune response; or 2) in minute quantities in vaccines manufactured using eggs, since eggs are rich in squalene and cholesterol. The FDA verified that none of the vaccines used during the Gulf War contained squalene as an adjuvant.

William Y. Ellis
Chief, Department of Chemical Information
Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Washington, DC 20307-5100

7 May,1999

Dear Sir:

This letter reports our preliminary findings on the determination of squalene in vials of an anthrax vaccine preparation.

Three vials of ANTHRAX VACCINE ADSORBED, Manufactured By MICHIGAN DEPARTMENT OF PUBLIC HEALTH, Lansing, Michigan, 48909, U.S. License No. 99, LOT FAV020, EXP 6 FEB 99, were received on 23 April 1999.

We have developed a sensitive, rapid assay method for squalene using high performance liquid chromatography. The assay specificity is based on chromatographic retention time and on the uv absorption characteristics of the analyte. The method sensitivity is ~0.7 nanogm squalene/10 microL injection, based on squalene in 2-propanol. The method linearity is 0.7 nanogm to 225 nanogm/10 microL injection with $r^2 = .999$, also based on squalene in 2-propanol. The method is currently undergoing validation.

We find no measurable amount of squalene in the vials. If any squalene were present, it would be less than 70 nanogm per 0.5 milliL vaccine preparation, which volume is the label dose.

We will prepare and submit our final report as soon as the study is completed.

Sincerely yours,

Peter Lim, Ph.D.
Principal Investigator
Catalysis and Anal. Chem. Dept.
Pure and Applied Phy. Chem. Div.

Ronald J. Sparrgord, Ph.D.
Assistant Principal Investigator
Catalysis and Anal. Chem. Dept.
Pure and Applied Phy. Chem. Div.

SRI International
333 Ravenswood Ave. • Menlo Park, CA 94025

JACK METCALF
2d District, Washington

COMMITTEE ON TRANSPORTATION AND INFRASTRUCTURE
SUBCOMMITTEES:
AVIATION
GROUND TRANSPORTATION

COMMITTEE ON SCIENCE
SUBCOMMITTEE:
ENERGY AND ENVIRONMENT

Congress of the United States
House of Representatives
Washington, DC 20515–4702

FINANCIAL SERVICES
SUBCOMMITTEES:
HOUSING
FINANCIAL INSTITUTIONS
DOMESTIC AND INTERNATIONAL MONETARY POLICY

CHAIR, REPUBLICAN HOUSING OPPORTUNITY CAUCUS

REPUBLICAN POLICY COMMITTEE

January 31, 2000

Jane E. Henney M.D., Commissioner
Food and Drug Administration
Room 1555
5600 Fishers Lane
Rockville, MD 20857

re: Department of Defense (DOD)
Report To Congress: Gulf War Illness
"Development and Validation of an Assay
To Test for the Presence of Squalene Antibodies"

Dear Commissioner Henney:

In its report provided to Congress this month, the DOD made the following statement in its Executive Summary: "The FDA verified that none of the vaccines used during the Gulf War contained Squalene as an adjuvant."

Unfortunately, the DOD report did not provide a site reference for their statement. Please provide copies of the written documents in which your verification was provided to the DOD.

Specifically, please provide answers to the following questions:

1. What vaccines were tested?
2. What lot numbers of those vaccines were tested?
3. Who did the testing?
4. Where was the testing done?
5. What specifically was being looked for during the testing?
7. Were any additional adjuvants identified during the testing?

Please respond within 14 days. Thank you for your attention to this matter.

Sincerely,

Jack Metcalf

Jack Metcalf

cc: Kathryn c. Zoon, Ph.D.

WASHINGTON OFFICE:
1510 Longworth HOB
Washington, DC 20515
(202) 225–2605

EVERETT OFFICE:
2930 Wetmore Avenue, #9E
Everett, WA 98201
(425) 252–3188
(800) 562–1385

BELLINGHAM OFFICE:
322 No. Commercial, #203
Bellingham, WA 98225
(360) 733–4500

DEPARTMENT OF HEALTH & HUMAN SERVICES

MAR 22 RECD

RECEIVED
Food and Drug Administration
Rockville MD 20857
MAR 28 2000

MAR 20 2000

The Honorable Jack Metcalf
House of Representatives
Washington, D.C. 20515-4702

Dear Mr. Metcalf:

Thank you for your letter dated January 31, 2000, addressed to Dr. Jane E. Henney, requesting information from the Food and Drug Administration (FDA) concerning squalene and vaccines used during the Gulf War. We apologize for the delay in responding.

Your letter referenced a Department of Defense (DOD) Report to Congress which you indicated had included the statement that "The FDA verified that none of the vaccines used during the Gulf War contained squalene as an adjuvant." Your letter requested both that verification to DOD and responses to a number of questions. FDA was unfamiliar with the DOD report you cited. On March 9, Ms. Jarilyn Dupont of my staff discussed this with Ms. Norma Smith of your district office and she provided FDA with the DOD Executive Summary referred to in your letter. In reviewing the DOD Executive Summary, it appears that the statement DOD made was in reference to a statement contained in a report from the Senate Special Investigation Unit (SIU) of the Senate Veterans' Affairs Committee which conducted a comprehensive review of Gulf War illnesses. That report indicated that the FDA verified that none of the vaccines used during the Gulf War contained squalene as an adjuvant. (*Report of the Special Investigation Unit on Gulf War Illnesses*, page 123, footnote 331).

In fact, FDA did verify to the Senate Special Investigations Unit on July 23, 1997, in a telephone conversation with Committee staff of the SIU, not with DOD, that neither the licensed vaccines known to be used in the Gulf War, nor the one investigational product known to have been used, contained squalene as an adjuvant in the formulations on file with FDA. FDA also has provided this information, and the information provided below, to the General Accounting Office (GAO) as part of an audit on squalene and Gulf War illness.

Currently, the only adjuvant in licensed vaccine formulations are aluminum compounds. Squalene, an intermediate in the

biosynthesis of cholesterol, is not approved for use as an adjuvant in licensed vaccines. Vaccines are not routinely tested for the presence or absence of squalene by the manufacturer or by FDA's Center for Biologics Evaluation and Research (CBER). Manufacturers perform specific tests as outlined in their license application. The tests for Anthrax Vaccine Adsorbed include Sterility, General Safety, Potency, Aluminum, Formaldehyde, and Benzethonium Chloride. Samples for the Anthrax lots and corresponding protocols containing the test results are submitted to CBER. CBER has the option to perform additional testing on lots submitted for lot release.

Very limited testing of Anthrax Vaccine, Adsorbed, conducted by CBER in 1999 determined that there were only trace amounts of squalene in the lots tested. After an article appeared in the May 1999 issue of Vanity Fair entitled "The Pentagon's Toxic Secret," CBER tested in its laboratories the two lots mentioned in the article (FAV020 and FAV030) for squalene. Three other Anthrax lots (FAV038, FAV043, FAV047) and two other lots of other bacterial vaccines (Wyeth Diphtheria and Connaught Tetanus) containing alum adjuvants were randomly selected for comparative purposes. Due to the inability to detect trace amounts of squalene parts per million, CBER developed a test to detect the substance in parts per billion. The trace amounts of squalene were determined by gas chromatography with flame ionization detection. The squalene content of the lots was determined to be in the level of low parts-per-billion and was comparable to levels determined in three other lots of the anthrax vaccine and the other biological products that were tested. In addition to squalene, lots FAV020 and FAV030 were also tested for aluminum, formaldehyde and benzethonium chloride.

We trust this information responds to your concerns. If we may be of any further assistance, please contact us again.

Sincerely,

Melinda K. Plaisier
Associate Commissioner
of Legislation

BAYLOR
COLLEGE OF
MEDICINE

Department of Immunology
One Baylor Plaza, BCMM-M929
Houston, TX 77030-3498
Tel: 713-798-6054
Fax: 713-798-3700

September 22, 2000

Congressman Jack Metcalf
2930 Wetmore Avenue, Suite 9-E
Everett, WA 98201

Dear Congressman Metcalf:

As you know, squalene is not approved for use as an immune adjuvant, however, there is evidence that very small amounts of the Anthrax Vaccine given to Gulf War participants contained this compound.

The tests done by SRI International were performed using a fairly sensitive technique called High Pressure Liquid Chromatography(HPLC). This technique is commonly used to find trace chemicals of drugs in a test specimen compared to a control specimen. However, as I understand this case, a much more sensitive test using gas chromatography, which instead of examining the test specimen as a liquid, vaporizes it which makes it a much more sensitive technique, found low levels of squalene in Anthrax vaccine samples.

The real issue is whether squalene in parts per billion was added to the vaccine preparations given to the military, as well as whether this concentration of squalene could alter the immune response.

More research needs to be done to answer these questions, but it is possible that very small amounts of a biologically active product could induce an immune response, either to the molecule itself or it could boost immune responses to other agents in the mixture. In any case, the discrepancy between the SRI test and that done by CBER needs to be investigated.

Sincerely,

Dorothy E Lewis

Dorothy E. Lewis, Ph.D.
Associate Professor of Immunology

DEL/tfs